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Habituation in the jumping spider

Trite planiceps

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Abstract

The ability to filter out repetitive, irrelevant, background stimuli prevents the nervous system from being flooded with information. However, animals must be particular to which stimuli they attend to and those that they ignore, as mistakes could have a high cost. One of the most fundamental forms of learning is habituation. Habituation is a form of non-associative learning, meaning that it is not linked with any other stimuli - it is simply the repetition of the same stimulus. I explored several characteristics of habituation with the New Zealand jumping spider *Trite planiceps*. These salticids are highly visual, and I used a visual stimulus in all experiments, as moving visual stimuli trigger a readily observable optomotor response. Firstly, I exposed *T. planiceps* to a repetitive visual stimulus, either in alternating or random fashion, and found that after only fifteen repetitions the orientation responses had dropped below 50%. I then explored the effect of interstimulus interval (ISI), using either an ISI of 10 s or 20 s between stimulus presentations. *T. planiceps* showed a significant difference in response decrement to repeated visual stimulation between the two ISIs, as found in other arthropods. Finally, I tested dishabituation, which is the recovery of the habituated response after the presentation of a novel stimulus, using a double air puff as the novel stimulus. Surprisingly, I got only a small response from *T. planiceps*, which was quite unlike the comparative literature of dishabituation on arthropods. I explain the possible reasons for this response in detail. While my data suggest that *T. planiceps* does habituate to repeated presentation of a visual stimulus, further testing is required before firm conclusions can be made.

Chapter 1.
Introduction

"Nevertheless the difference in mind between man and the higher animals, great as it is, certainly is one of degree and not of kind" (cited in Darwin 1874).

Arthropod cognition

Early work in insect learning focused on simple associative processes with colour, patterns and places (Menzel 1993). The view that insects and other arthropods, such as spiders, were entirely governed by innate hard-wired routines was supported by behavioural observations on the digger wasp *Sphex ichneumoneus*. When this wasp was interrupted during its offspring provisioning behaviour the wasp would continue to repeat the routine behaviour well beyond what was necessary (Wooldridge 1963, p. 82, as cited in Dennett 1984, p. 11). This hardwiring concept was based on two assumptions: (1) that arthropod brains are too small for complex behaviours (Dukas 2008); (2) that their lifespan is too short for learning to be beneficial (Alexander *et al.* 1997). However, there are flaws in these assumptions. Many arthropods are, in fact, extremely long-lived. For example, many species of spiders may live up to thirty years or more (Costa and Pérez-Milles 2002) and many insects live over a year (Danks 1992). Additionally, we shouldn't dismiss the abilities of arthropods based on brain size alone. Compared with smaller brains, large brains often only show quantitative improvements, especially in areas of sensory or motor processing, such as higher sensitivity or finer resolution (Chittka and Niven 2009). Also, as learning could be a requirement for coping with environmental unpredictability (Alcock 2005), the opportunity of advantageously using learned knowledge is still worthwhile, regardless of the expected life span of the animal (Dukas 2008).

Rather than simply being present or absent, cognitive ability is likely to lie on a continuum, with animals at one end of the spectrum performing principally innate behaviour with little learning capacity, and animals at the other end being capable of elaborate information processing and decision-making (Jackson and Cross 2011). Complex cognitive abilities commonly attributed to vertebrates, such as rule learning, categorisation and concept formation, have been demonstrated in insects and other arthropods, including spiders (Menzel

2012; Jackson and Cross 2011). It is possible that these behaviours are adaptive strategies of economising memory in small brains (Srinivasan 2006).

Learning from experience has been shown to influence the behaviour of spiders in many aspects of their lives, such as foraging (Jackson and Wilcox 1993), mate choice (Hebets 2003) and intraspecific conflict (Whitehouse 1997). Hénaut (2013) recorded an excellent example in the field of single-trial learning by the web building spider *Nephila clavipes.* Ants (*Ectatomma tuberculatum*) frequently fall into the webs of these spiders, but they have an unpleasant bite. After experiencing one ant bite, spiders subsequently avoided ants that fell on their webs (Hénaut 2013).

However, it is among the jumping spiders (family Salticidae) where cognitive ability seems especially well developed (Jackson and Cross 2011). Typically measuring less than one centimetre in body length, these stocky salticids do not build webs. Instead, salticids build a silken retreat (nest) in which they sleep at night. During the day, these highly visual salticids are active, often ambushing or actively stalking visually-detected prey (Foelix 2011). Learning has been particularly well-studied in salticids. For example, Skow and Jakob (2005) showed how after exposure to distasteful milkweed bugs (*Oncopeltus fasciatus*) the salticid *Phidippus princeps* learned to avoid the bugs. Interestingly, this avoidance behaviour was 'reset' when the environment was changed, indicating that contextual cues were important for expression of this learned behaviour. This makes sense, because the palatability of the bug depends on which species of plant it feeds on and the distribution of milkweed plants varies across environments. The results by Skow and Jakob (2005) should thus make it unsurprising that salticids have also been successfully trained. Jakob and colleagues (2008) trained *P. princeps* to associate colour cues with prey in a t-maze, while recently Peckmezian and Taylor (2015) trained *Servaea incana* to associate a dark area with subsequent electric shock.

Being able to learn from experience can be linked to learning by trial and error, which is considered more difficult than associative learning because the reward does not always follow the action, especially if a lot of error is involved. The salticid genus *Portia* specialises on eating other spiders, and often uses a combination of web plucking techniques using its legs, body and palps to evoke a response from a resident web-building spider, which it then attacks and consumes. *Portia* is well known to learn which particular plucking routines are successful at luring resident spiders toward them slowly (a quick approach could be

dangerous for *Portia*) through trial and error, remembering the order, the type of plucking that was successful, and then later using the same combination on another web of the same species (e.g., Jackson and Carter 2001; Jackson and Nelson 2011). *Portia* is also able to distinguish which combinations work best for each species of web-building spider targeted (Jackson and Wilcox 1998). This ability to learn web-plucking routines by trial and error has also been shown in other web-invading salticids, including *Brettus adonis, Brettus albolimbatus, Cyrba algerina, Cyrba ocellata,* and *Cyrba simoni* (Jackson 2002). Another example of learning by trial and error is shown by *Portia fimbriata*, which hunts the salticid *Euryattus* sp. when the females build nests within a rolled up leaf in anticipation of mating. When a male *Euryattus* find a female's nest it will rock the leaf violently back and forth to signal to the female to come out of the nest to mate. *P. fimbriata* mimics these signals, luring females out of the leaf nest and then pouncing on the emerging female (Jackson and Wilcox 1998).

In order to hunt web-building spiders, *Portia* may take long detours to reach an advantageous area in which to pounce on the potentially dangerous spider (Tarsitano and Jackson 1997). Tarsitano and Jackson (1997) tested six detour routes with increasing complexity with *Portia fimbriata,* a species that has been recorded to take long and complicated detours in the wild. They found that *Portia* could distinguish between correct and incorrect routes even when the correct route began in the opposite direction of the prey. Tarsitano and Jackson (1994) also investigated detouring behaviour in *Trite planiceps*, the salticid species studied in this thesis. They found that *T. planiceps* were able to complete detours, but only in the presence of a moving target (a live fly) (Tarsitano and Jackson 1994). To be able to choose the correct route requires the knowledge of how all the components of the route connect to the prey, showing hunting strategies not dissimilar to that of vertebrates (Tarsitano and Jackson 1997). Additionally, as these detours require salticids to lose sight of the prey in order to get to it, this work raises questions of mental maps or mental representations in salticids.

These previous findings pave the way for further research on the cognitive capabilities of small-brained animals that once were, and some still believe today, thought incapable of learning. It is these stepping stones of cognitive ability which make work on invertebrates so interesting. The field of testing animal cognition is flawed in the sense that the majority of theories defining 'cognitive behaviour' are based on what we consider cognitive in ourselves; concept learning, problem solving, learning by trial and error, and language, to name a few.

However, our understanding of cognition in arthropods is expanding what we consider cognitive behaviour, as well as which animals, and to what extent, express 'surprising' cognitive ability. It was my knowledge of work on salticids that inspired me to conduct research of my own on these salticids - in this case looking at habituation, a rather more basic form of learning, which is an area that has been largely overlooked in salticid research.

Study species

Like all arthropods, salticids are limited in size by their exoskeleton. This also means that salticid eyes cannot be very large, although they achieve what a larger eye can do through 'division of labour'. Salticids use four separate pairs of eyes (Fig. 1.1) which combined do the tasks of high-resolution vision (high spatial acuity), motion vision, and colour vision (Land 1971; Zurek and Nelson 2012). These four eye pairs consist of a large pair of forward-facing anterior median (AM) or 'primary' eyes and the anatomically distinct three pairs of 'secondary' eyes: the anterior lateral (AL) eyes, which are forward facing, and the posterior median (PM) and the posterior lateral (PL) eyes, which are sideways facing and smaller than the AM and AL eyes.

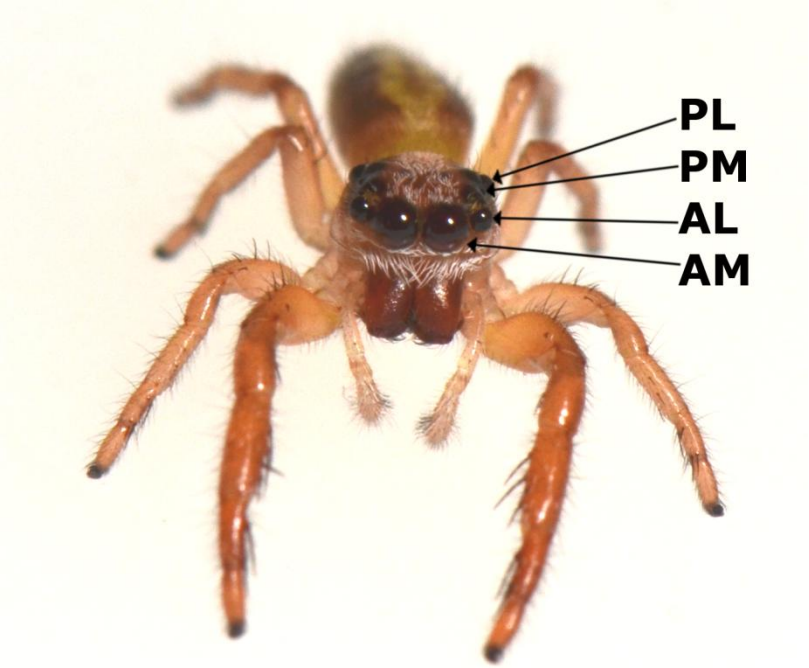

Fig. (1.1). Juvenile *Trite planiceps* illustrating the anterior median (AM), anterior lateral (AL), posterior median (PM) and posterior lateral (PL) eyes.

The main role of secondary AL, PL and PM eyes are generally considered as motion detection. Stimuli appearing only in the field of view of the AL eyes will mediate an orientation response (Land 1971; Duelli 1978). This orientation response has the goal of bringing an object identified by the secondary eyes into the field of view of the high spatial acuity AM eyes for further inspection in greater detail (Zurek and Nelson 2012). In my thesis

I have specifically stimulated only the AL eyes because of their ability to elicit an easily seen orientation response.

The salticid genus *Trite* is composed of 18 species which inhabit New Zealand, Australia and the southwest pacific (Zabka 1988; Berry *et al.* 1997; Vink *et al.* 2011). Commonly known as the black-headed jumping spider, *Trite planiceps,* the species used for my research, is a salticid endemic to New Zealand (Forster and Forster 1973). Apart from its mating behaviour (Jackson 1986; Taylor and Jackson 1999), and ability to perform detours (Tarsitano and Jackson 1994), little is known about its behaviour.

Often found inside rolled up New Zealand flax (*Phormium tenax*) and cabbage tree (*Cordyline* spp.) leaves (Forster and Forster 1973), *T. planiceps* has a much longer (10-13 mm) body than other New Zealand species of *Trite* (Vink *et al.* 2011). This salticid has a distinguishable black head and yellow to dark green and black abdomen (Fig. 1.2). *T. planiceps* are insectivorous generalists, and grass moths (*Orocrambus flexuosellus*) are readily captured and eaten by *T. planiceps* (Moss *et al.* 2006). *T. planiceps* primarily use vision for capturing prey, but are also skilled hunters in complete darkness in the absence of visual cues (Forster 1982; Taylor 1995). *T. planiceps* is parasitized by the pomplilid wasp *Epipompilus insularis* and is known to be predated by conspecifics, the earwig *Forficula auricularia*, the salticids *Clubiona cambridgei, Cheiracanthium stratioticum* and *Zelanda erebus* (Vink *et al*. 2011), as well as the local introduced bird populations such as the common blackbird (*Turdus merula)* and the house sparrow (*Passer domesticus*).

Fig. (1.2). Adult male (left) and female (right) *Trite planiceps*. Note the difference in size of the palps and length of the first pair of legs. Also, the male appears to have a tubercle or 'bump' on outer curve of the fang (red arrows).

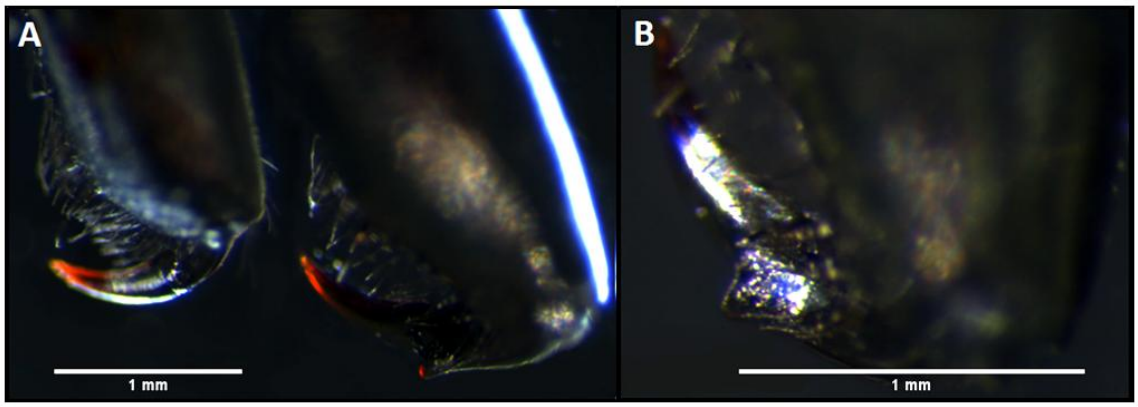

Fig. (1.3). *Trite planiceps* mouthparts. A. Close up of a male with tubercle on outer curve of fang. B. Comparison of the tubercle with the same male of the pre-adult moult and then the adult moult. Photos taken using a Leica DFC310 FX camera.

When I began working with this species, I noticed that the adult male *T. planiceps* had a tubercle, or bump, on the outside curve of the fang. Vink and collegues (2011) first described this tubercle among male *T. planiceps* and, exploring further, I found that this tubercle only becomes apparent in the very last moult of the adult male. I used a Leica DFC310 FX camera and Leica application suite software to image the fangs (Fig 1.3). However, due to the dark colour of the fangs it was difficult to see the tubercle in great detail even under florescence.

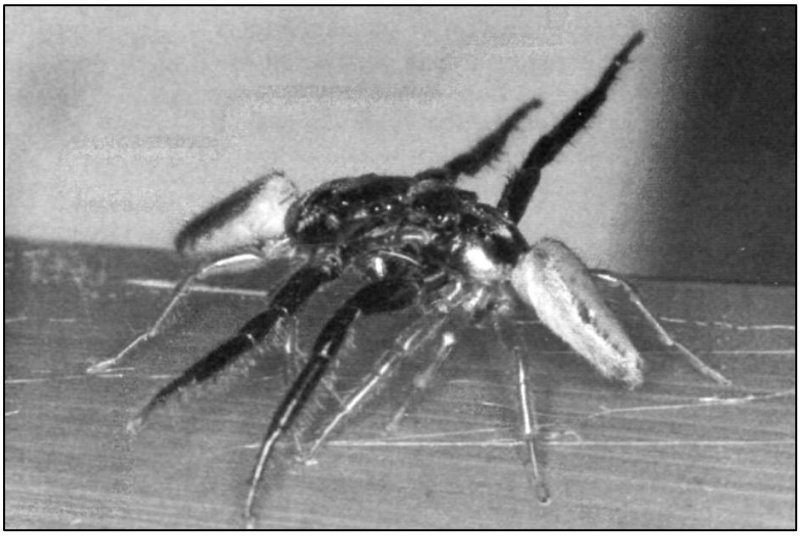

Fig. (1.4). Adult male *Trite planiceps* embracing and duelling by pushing their fangs against each other and waving their first pair of legs up and down. Source: Taylor and Jackson (1999).

In an attempt to determine the function of this tubercle I recorded the courtship and mating behaviour of *T. planiceps* (3 males and 3 females) with a Casio® ExFH25 high speed camera (60 frames per second). However, upon careful scrutiny of this footage I was only able to conclude that male *T. planiceps* did not use their fangs at any time during courtship or mating. My conclusion is that perhaps the tubercle is used as a method of securing another rival male's fangs during embracing and duelling (Fig. 1.4), first described in *T. planiceps* by Taylor and Jackson (1999). During embracing and duelling, rival males push their fangs against each other and wave their first legs up and down in a threatening manner. I attempted to replicate this behaviour but did not succeed - potentially because the breeding season was over. Ultimately, I was unable to explore this avenue further due to time restraints.

Thesis structure

This thesis consists of five chapters, with the introduction as Chapter 1.

In Chapter 2, I introduce the theory of habituation as background for my research question, which was whether *T. planiceps* habituates to repetitive stimulus presentations. Here, I exposed *T. planiceps* to a repetitive visual stimulus to see if the salticids would reduce their orienting response rate in a manner consistent with habituation.

In Chapter 3 I explored one of the characteristics of habituation, which is the effect of interstimulus interval (ISI) durations on habituation. By altering the time between stimuli in two groups of 10 s and 20 s ISI I investigated if the response decrement rate to repetitive stimuli was affected by shorter ISI.

In Chapter 4 I explored dishabituation with *T. planiceps.* By introducing a novel stimulus during habituation testing, the response rate should recover to the pre-habituated rate.
I tested two separate intervals of dishabituation, one at predetermined intervals and one only after four failed responses from the salticid.

Finally, I discuss my findings within a broader framework in Chapter 5. I relate my research back to the literature and compare the results I gained from *T. planiceps* to those found in other arthropods.

References

Alcock J. 2005. *Animal Behavior,* Eighth Edition. Sunderland, MA: Sinauer.

Alexander, R. D., Marshall, D. C. & Cooley, J. R. 1997. Evolutionary perspectives on insect mating. In *The evolution of mating systems in insects and arachnids*, ed. JC Choe, BJ Crespi, 4-31. New York: Cambridge University Press.

Berry, J.W., Beatty, J.A., Prószyński, J. 1997. Salticidae of the Pacific Islands. III. Distribution of seven genera, with descriptions of nineteen new species and two new genera. *Journal of Arachnology,* **26**, 149-189.

Chittka, L. & Niven, J. 2009. Are Bigger Brains Better? *Current Biology*, **19**, 995-1008.

Costa, F. G. & Pérez-Miles, F. 2002. Reproductive biology of uruguayan theraphosids (Araneae, Mygalomorphae). *Journal of Arachnology*, **30**, 571-587.

Darwin, C. 1874. *The Descent of Man and Selection in Relation to Sex,* Second Edition. John Murray, London.

Danks, H. V. 1992. Long life cycles in insects. The Canadian Entomologist, **124**, 167-187.

Dennett, D.C. 1984. *Elbow room: the varieties of free will worth wanting*. Cambridge, MA: MIT Press.

Dukas, R. 2007. Evolutionary biology of insect learning. *Annual Review of Entomology,* **53**, 145-160.

Duelli, P. 1978. Movement detection in the posterolateral eyes of jumping spiders (*Evarcha arcuata*, Salticidae). *Journal of comparative physiology*, **124**, 15-26.

Foelix, R.F. 2011. *Biology of Spiders,* Third Edition. Oxford University Press, New York.

Forster, L. M. 1982. Non-visual prey-capture in *Trite planiceps*, a jumping spider (Araneae, Salticidae). *Journal of Arachnology*, **10**, 179-183.

Forster, R. R. and Forster, L . J. 1973. *New Zealand Spiders — an introduction*. 254 pp. Collins, Auckland. London .

Hénaut, Y., Machkour-M'Rabet, S. & Lachaud, J.-P. 2013. The role of learning in risk-avoidance strategies during spider–ant interactions. *Animal Cognition*, **17**, 185-195.

Jackson, R. R. & Carter, C. M. 2001. Geographic variation in reliance on trial-and-error signal derivation by *Portia labiata*, an araneophagic jumping spider from the Philippines. *Journal of Insect Behavior*, **14**, 799-827.

Jackson, R. R. & Nelson, X. J. 2011. Reliance on trial and error signal derivation by Portia africana, an araneophagic jumping spider from East Africa. *Journal of Ethology*, **29**, 301-307.

Jackson, R. R. 2002. Trial-and-error derivation of aggressive-mimicry signals by *Brettus* and *Cyrba*, spartaeine jumping spiders (Araneae: Salticidae) from Israel, Kenya, and Sri Lanka. *New Zealand Journal of Zoology*, **29**, 95-117.

Jackson, R. R. 1986. Cohabitation of males and juvenile females: a prevalent mating tactic of spiders. *Journal of Natural History*, **20**, 1193-1210.

Jackson, R. R. & Cross, F. R. 2011. Spider cognition. *Advances in Insect Physiology*, **41**, 115.

Jackson, R. R. & Wilcox, R. S. 1998. Spider-Eating Spiders. *American Scientist*, **86,** 350-357.

Jakob, E. M., Skow, C. D., Haberman, M. P. & Plourde, A. 2007. Jumping spiders associate food with color cues in a T-maze. *Journal of Arachnology*, **35**, 487-492.

Land, M. 1971. Orientation by jumping spiders in the absence of visual feedback. *Journal of Experimental Biology*, **54**, 119-139.

Menzel, R. 1993. Associative learning in honey bees. *Apidologie*, **24**, 157–168.

Menzel, R. 2012. The honeybee as a model for understanding the basis of cognition. *Nature Reviews Neuroscience*, **13**, 758-768.

Moss, R., Jackson, R. R. & Pollard, S. D. 2006. Hiding in the grass: background matching conceals moths (Lepidoptera: Crambidae) from detection by spider eyes (Araneae: Salticidae). *New Zealand Journal of Zoology*, **33**, 207-214.

Peckmezian, T. & Taylor, P. W. 2015. Electric shock for aversion training of jumping spiders: Towards an arachnid model of avoidance learning. *Behavioural Processes*, **113**, 99-104.

Skow, C. D. & Jakob, E. M. 2006. Jumping spiders attend to context during learned avoidance of aposematic prey. *Behavioral Ecology*, **17**, 34-40.

Srinivasan, M.V. 2006. Honeybee vision: in good shape for shape recognition. *Current Biology*. **16**, 58–60.

Taylor, P. W. 1995. Jumping spiders alternative turns in the absence of visual cues. *International Journal of Comparative Psychology*, **8**, 69-76.

Taylor, P. W. & Jackson, R. R. 1999. Habitat-adapted communication in *Trite planiceps*, a New Zealand jumping spider (Araneae, Salticidae). *New Zealand Journal of Zoology*, **26**, 127-154.

Tarsitano, M. S. & Jackson, R. R. 1994. Jumping spiders make predatory detours requiring movement away from prey. *Behaviour*, **131**, 65-73.

Tarsitano, M. S. & Jackson, R. R. 1997. Araneophagic jumping spiders discriminate between detour routes that do and do not lead to prey. *Animal Behaviour*, **53**, 257-266.

Vink, C., Dupérré, N. & McQuillan, B. 2011. The black-headed jumping spider, *Trite planiceps* Simon, 1899 (Araneae: Salticidae): redescription including cytochrome c oxidase subunit 1 and paralogous 28S sequences. *New Zealand Journal of Zoology*, **38**, 317-331.

Whitehouse, M. E. 1997. The benefits of stealing from a predator: foraging rates, predation risk, and intraspecific aggression in the kleptoparasitic spider *Argyrodes antipodiana*. *Behavioral Ecology*, **8**, 665-667.

Zabka, M. 1988. Salticidae (Araneae) of Oriental, Australian and Pacific Regions, III. *Annales zoologici, Warszawa*, **41**, 421-479.

Zurek, D. B. & Nelson, X. J. 2012. Saccadic tracking of targets mediated by the anterior-lateral eyes of jumping spiders. *Journal of Comparative Physiology*, **198**, 411-417.

Chapter 2.

Do jumping spiders habituate to repetitive visual stimuli?

Detection of a predator prior to an attack is critical for animal's survival if it is detected in time for the potential prey to flee (Yamawaki and Ishibashi 2014). This apparently trivial statement underlies remarkable complexity, however, as the nervous system must process sensory information, categorise the information, and based on that categorisation, make a decision to flee, ignore, or approach the stimulus (e.g., in the case of the stimulus being a potential mate). However, nervous systems show limitations in their ability to maintain high quality information processing for extended periods of time (Dukas and Clark 1995). Therefore, animals must be particular to which stimuli they respond and which should be ignored, as the consequences for a mistake could be fatal (Christensen *et al.* 2008). This is likely to be most evident in animals with small nervous systems, such as insects and spiders, whose 'brains' are quantitatively reduced when compared to vertebrates (Chittka and Niven 2009).

The most fundamental form of adaptation to experience is habituation (Dong and Clayton 2009), which is a decrease in the strength (including complete cessation) of a response after repeated presentations of a stimulus which elicits the response (Mazur 2006). By allowing an animal to distinguish biologically 'irrelevant' background stimuli, such as perhaps the shadow of leaves moving in the wind, from biologically important stimuli, such as predator, prey or mate detection, this process protects the organisms' nervous system from being flooded with irrelevant information (Klingner *et al.* 2014). Habituation does not require conscious motivation or awareness and does not involve sensory adaptation or fatigue (Rankin *et al.* 2009). For example, response decrements resulting from very rapid stimulation (e.g. 500 Hz), trauma, growth or aging are not classified as habituation (Thompson and Spencer 1966). Habituation and has been demonstrated – at least to some extent - in most animals, from the protozoan *Stentor* (Wood 1988) to humans (Geer 1966).

Unlike other response decrements, habituation is defined by ten characteristics first categorised by Thompson and Spencer (1966), Groves and Thompson (1970) and later edited by Rankin and colleagues (Rankin *et al.* 2009). These characteristics are:

1. Repeated application of a stimulus results in a progressive decrease in response ('response decrement').
2. If the stimulus is withheld for some period after a response decrement to it, the response shows spontaneous recovery to the original stimulus.
3. After multiple bouts of habituation training and spontaneous recovery events, the response decrement becomes successively more pronounced or rapid.
4. More frequent stimulation (shorter interstimulus interval) results in a faster or more pronounced response decrement and also results in more rapid spontaneous recovery.
5. The less intense the stimulus the more rapid or pronounced the response decrement.
6. The response decrement may reach 'below zero' habituation where, if the habituation builds up for long enough, the spontaneous recovery may be delayed.
7. Within the same sensory modality the response decrement shows a degree of stimulus specificity, but there may be some generalisation between similar stimuli.
8. Once the animal has habituated to the stimulus the presentation of a different stimulus results in the recovery of the habituated response. This is called dishabituation.
9. The dishabituation effect of the stimulus also shows a gradual decrement in response with repeated presentations.
10. Habituation can occur for a period of days, weeks or months. This form of habituation is called long-term habituation.

These ten characteristics apply to all the successful habituation studies (Rose and Rankin 2001). Because these studies encompass a wide range of animal taxa, this supports the hypothesis that habituation is critical for survival, as it has not been altered significantly through evolution, and may consist of cellular mechanisms which are conserved across phylogenies (Rose and Rankin 2001).

Non-associative stimuli are those in which stimuli are not linked with other stimuli. Habituation is considered only in the context of non-associative stimuli, and as such is often considered the simplest form of learning (Christensen *et al.* 2008). Therefore, it commonly thought that to properly understand other, more complex, forms of learning habituation must first be completely understood, even at the level of the molecular pathways within cells

(Rankin *et al.* 2009; Rose and Rankin 2001). However, the idea of habituation being a straightforward process is somewhat simplistic and to this day remains an unsolved problem in neuroscience (Ramsawari 2014) despite over a century of work on the subject (Thompson 2009). This is, in part, because while non-associative stimuli are the most basic sort of stimuli with which to test animals, the actual process of habituation necessitates neural systems to constantly evaluate incoming stimuli to filter out those that are not important while retaining some of that information. This process is achieved through a collection of cellular mechanisms which are yet to be completely identified - hardly making habituation a clear cut pathway (Rankin *et al.* 2009). Nevertheless, the goal of understanding how nervous systems produce behaviour at least appears tractable in invertebrates (Edwards *et al.* 1999). Invertebrate nervous systems generally contain only several thousand (or hundred thousand) neurons (as opposed to billions in many vertebrates), with some of the cells being large and easily identifiable, making them excellent candidates for research into learning and memory (Martinez and Kesner 1998). Research using invertebrates therefore may provide the necessary platform from which to begin to fathom how nervous systems facilitate and change due to learned behaviour (Martinez and Kesner 1998).

It remains unknown who first coined the term "habituation", but interest in the subject rapidly expanded around the early twentieth century (Thompson 2009). Gee (1913) was the first to document how the leech (*Hirudo medicinalis*) would stop responding to a shadow overhead or a jarring motion after multiple presentations (cited in Boulis and Sahley 1988). Holmes (1912) used the terms "dishabituation" and "dehabituation" in his work on the sea urchin (*Arbacia punctulata*) when referring to the renewal of the original response. In contrast, Humphrey (1933) used "fatigue" and "negative adaptation", and Harris (1943) added the terms "extinction" and "stimulatory inactivation" to the vocabulary (cited in Thompson 2009).

One renowned study on habituation was by Sharpless and Jasper (1956) who repeated brief tones to a sleeping cat and recorded cortical arousal through electroencephalography (EEG). Sharpless and Jasper (1956) found that: arousal to the stimulus would eventually decrease until it was no longer measureable using EEG; arousal would exhibit spontaneous recovery after the stimulus was removed for a time; the arousal response showed dishabituation, or response renewal. Interestingly, once the sleeping cat was habituated to a 500 cycles per second (cps) tone, the presentation of a 600 cps tone would elicit no reaction, yet the

presentation of a 1000 cps tone would elicit a rapid arousal response, indicating the ability to distinguish between the two (500 and 1000) tones. Following this study awareness of habituation as a fundamental form of behavioural plasticity was established (Thompson 2009).

Working on unicellular organisms at another end of the spectrum, Wood (1988) examined the ciliate protozoan *Stentor coeruleus. S. coeruleus* contracts in response to a mechanical stimulus but ceases to respond to a repetitive mechanical stimulus, becoming progressively more unresponsive (Table 2.1). While habituation has been investigated in unicellular organisms it has been more widely studied among invertebrates, where there are numerous studies that have addressed at least some of the ten characteristics of habituation. These are summarised in Table 2.1, and a few detailed examples are described below.

The nematode *Caenorhabditis elegans* is a model multicellular organism in which to investigate the cellular and molecular basis of behaviour because it has approximately 302 neurons (Rose and Rankin 2001). This extraordinarily tractable number gives researchers the opportunity to carefully investigate the nematode's neurons and synaptic connections (Martinez and Kesner 1998) and White and colleagues (1986) have mapped the nematode's entire nervous system. However, it wasn't until Rankin and colleagues (1990) began to look for a model organism to investigate learning and memory that habituation was tested in *C. elegans. C. elegans* responds to the mechanical stimulus of a tap to a petri dish (in which it is housed) by rapidly swimming backwards (Rose and Rankin 2001). Measured as the average distance swimming backwards, this so-called 'tap withdrawal response' has been shown to habituate (Rose and Rankin 2001).

Another well-studied example of habituation and spontaneous recovery occurs in the crayfish. *Procambarus clarkii* exhibit an escape reflex called the LG (lateral giant) escape reflex when a mechanical stimulus is applied to the end of the abdomen. Here, the LG axons are activated and elicit flexing in the rostral (anterior) segments of the abdomen, creating a swift propulsive thrust upwards, and away from the stimulus. The LG axons receive combined information from the primary afferent (sensory) neurons and sensory interneurons in the abdomen, triggering large excitatory synapses with giant motor neurons which stimulate the rostral abdomen phasic flexor muscles, propelling the animal through the water (Mittenthal and Wine 1973; Wine and Krasne 1982). However, after repeated mechanical

stimulation the escape response is no longer activated unless the stimulation is stopped for a time, whereupon further stimulation will trigger an escape response (Krasne 1969). This phenomenon can also be seen in *Aplysia californica*, a mollusc commonly known as the sea hare, which has become the iconic animal for research on non-associative learning thanks to its large and easily identifiable neurons (Pinsker *et al.* 1970). The two main reflexes which have been studied in *Aplysia* for habituation are the gill-withdrawal and siphon-withdrawal reflexes. These reflexes elicit withdrawal of the organ when given mechanical stimulation (Pinsker *et al.* 1970, Kupfermann *et al.* 1974). *Aplysia* appear to show most of the characteristics for habituation including: interstimulus interval spontaneous recovery, dishabituation and long term habituation (Table 2.1).

Research on habituation in vertebrates is gaining popularity as a means of testing development. *Aplysia* show an increase in their response decrement to a repetitive mechanical stimulus as they mature from juvenile to the adult stage (Rankin and Carew 1987).
Recent work involves testing the habituation of gaze following behaviour (when an animal tracks the gaze direction of another to a location in space) in young ravens (*Corvus corax*). Schloegl and colleagues (2007) tested gaze following with raven chicks and found that at eight weeks they would follow a human looking up but would not follow a human gazing behind a barrier until they were eight months old. Interestingly, the ravens habituated to repetitive look ups but did not habituate to repetitive looking behind a barrier and would continuously explore the area (Schloegl *et al.* 2007).

Extensively studied in humans is the orienting reflex. The orienting reflex was first described in 1910 by Pavlov as the "what is that?" reflex (cited in Barry 2009). Sokolov (1960, 1963, cited in Barry 2009) identified the orienting reflex as an important part of perceptual functioning, as it points the attention of the organism to events in the surrounding area. Human infants c. 80 hours old have been shown to habituate to rattle sounds by decreasing localized head turning, and, as expected, also exhibit recovery in response rate after a dishabituating stimulus (Zelazo 1984). These results indicate the beginning of information processing and memory formation capacity in very young infants (Zelazo 1984).

Table 2.1. Comparison of habituation, interstimulus interval and dishabituation for research across invertebrates and unicellular organisms. Interstimulus interval and dishabituation are explained further Chapters 3 and 4. Numbers in parenthesis refer to references footnoted at the end of the table. *Rare case of interstimulus interval dependant dishabituation. **Unusual occurrence where the cuttlefish failed to dishabituate to the stimulus.

Animal	Phylum	Habituation	Stimulus	Interstimulus interval (ISI)	Spontaneous recovery ISI	Dishabituation	Stimulus	Other habituation characteristics
Protozoan *Stentor coeruleus*	Ciliophora	Yes [1]	Mechanical	Not tested	Not tested	Not tested	Not tested	Not tested
Protozoan *Spirostomum ambiguum*	Ciliophora	Yes [2]	Vibration	Not tested	Not tested	Yes [2]	Electric Shock	Spontaneous recovery after rest period [2]
Fly *Drosophila melanogaster*	Arthropoda	Yes [3]	Electric pulse	Yes [3] 2,5,10 Hz ISI	Not tested	Yes [3]	Air puff, Light flash	Spontaneous recovery after rest, sub-zero habituation, habituation of dishabituation [3]
Locust *Locust migratoria*	Arthropoda	Yes [4]	Looming object	Not tested	Not tested	Yes [4]	Change in trajectory	Not tested
Bumblebee *Bombus impatiens*	Arthropoda	Yes [5]	Visual pattern	Not tested	Not tested	Yes [5]	New visual pattern	Spontaneous recovery after rest [5]
Araneid spider *Cyclosa conica*	Arthropoda	Yes [6]	Vibration by tuning fork	Not tested	Not tested	Not tested	Not tested	Not tested
Salticid spider *Metaphidippus harfordi* and *Metaphidippus aeneolus*	Arthropoda	Yes [7]	Visual (rotational movement of stimulus)	Not tested	Not tested	Yes [7]	Removed paper ring platform	Spontaneous recovery after rest [7]
Crayfish *Procambarus clarkii*	Arthropoda	Yes [17][18]	Tactile [17] Looming object [18]	Yes [19] 1,5,20,60,300 s ISI	Yes	Yes [18]	Tactile	Spontaneous recovery after rest period, stimulus generalization [18]
Crab *Chasmagnathus granulatus*	Arthropoda	Yes [20]	Shadow overhead	Yes [20] 0, 171 s ISI	Not tested	Yes [21]	Light + Electric shock /Dark + Electric shock [21]	Long term habituation [20], Sensitization [21]
Jellyfish *Aurelia aurita*	Cnidaria	Yes [8]	Tactile	Yes [8] 2, 6 minute ISI	Not tested	Yes [8]	Shake	Spontaneous recovery, ISI dependant dishabituation * [8]
Leech	Annelida	Yes [9]	Light flash [9]	Yes [10]	Not tested	Yes [9]	Electric shock	Sensitization [9]

Hirudo medicinalis			Electric shock [10]	10,44,300 s ISI				Failure to habituate long term [9]
Worm *Caenorhabditis elegans*	Nematoda	Yes [11]	Tap	Yes [11] 2,10,30,60 s ISI	Yes	Yes [12]	Electric shock	Sensitization by a rapid set of taps [12]
Sea hare *Aplysia californica*	Mollusca	Yes [13]	Tactile	Yes [16] 3,10,30,100 s ISI	Yes	Yes [15]	Electric shock	Long term habituation and sensitization [14]
Cuttlefish *Sepia officinalis*	Mollusca	Yes [22]	Prawn /fish in tube	Not tested	Not tested	No** [22]	Free prawn	N/A
Squid *Lolliguncula brevis*	Mollusca	Yes [23]	Plastic model	Not tested	Not tested	Yes [23]	Noxious stimulus	Spontaneous recovery after rest, long term habituation, stimulus specificity [23]

[1] Wood, D. C. 1988. Habituation in *Stentor*: produced by mechanoreceptor channel modification. *The Journal of Neuroscience*, **8**, 2254-2258. **[2] Osborn, D., Blair, H. J., Thomas, J. & Eisenstein, E.** 1973. The effects of vibratory and electrical stimulation on habituation in the ciliated protozoan, *Spirostomum ambiguum*. *Behavioral Biology*, **8**, 655-664. **[3] Engel, J. E. & Wu, C.-F.** 1996. Altered habituation of an identified escape circuit in *Drosophila* memory mutants. *The Journal of Neuroscience*, **16**, 3486-3499. **[4] Gray, J. R.** 2005. Habituated visual neurons in locusts remain sensitive to novel looming objects. *Journal of Experimental Biology*, **208**, 2515-2532. **[5] Plowright, C. M. S., Simonds, V. M. & Butler, M. A.** 2006. How bumblebees first find flowers: Habituation of visual pattern preferences, spontaneous recovery, and dishabituation. *Learning and Motivation*, **37**, 66-78. **[6] George, W. & Peckham, E. G.** 1887. Some observations on the mental powers of spiders. *Journal of Morphology*, **1**, 383-419. **[7] Land, M.** 1971. Orientation by jumping spiders in the absence of visual feedback. *Journal of Experimental Biology*, **54**, 119-139. **[8] Johnson, M. C. & Wuensch, K. L.** 1994. An investigation of habituation in the jellyfish *Aurelia aurita*. *Behavioral and Neural Biology*, **61**, 54-59. **[9] Lockery, S., Rawlins, J. & Gray, J.** 1985. Habituation of the shortening reflex in the medicinal leech. *Behavioral Neuroscience*, **99**, 333. **[10] Boulis, N. M. & Sahley, C.** 1988. A behavioral analysis of habituation and sensitization of shortening in the semi-intact leech. *The Journal of Neuroscience*, **8**, 4621-4627. **[11] Rose, J. K. & Rankin, C. H.** 2001. Analyses of habituation in *Caenorhabditis elegans*. *Learning & Memory*, **8**, 63-69. **[12] Rankin, C. H., Beck, C. D. & Chiba, C. M.** 1990. *Caenorhabditis elegans*: a new model system for the study of learning and memory. *Behavioural Brain Research*, **37**, 89-92. **[13] Pinsker, H., Kupfermann, I., Castellucci, V. & Kandel, E.** 1970. Habituation and dishabituation of the GM-withdrawal reflex in *Aplysia*. *Science*, **167**, 1740-1742. **[14] Carew, T. J., Pinsker, H. M. & Kandel, E. R.** 1972. Long-term habituation of a defensive withdrawal reflex in *Aplysia*. *Science*, **175**, 451-454., **Pinsker, H. M., Hening, W. A., Carew, T. J. & Kandel, E. R.** 1973. Long-term sensitization of a defensive withdrawal reflex in *Aplysia*. *Science*, **182**, 1039-1042. **[15] Carew, T. J., Castellucci, V. F. & Kandel, E. R.** 1971. An analysis of dishabituation and sensitization of the gill-withdrawal reflex in *Aplysia*. *International Journal of Neuroscience*, **2**, 79-98. **[16] Byrne, J. H.** 1982. Analysis of synaptic depression contributing to habituation of gill-withdrawal reflex in *Aplysia californica*. *Journal of Neurophysiology*, **48**, 431-438. **[17] Wine, J.J. & Krasne, F.B.** 1982. The Biology of Crustacea Neural Integration and Behaviour (Vol. 4) (Sandeman, D.C. and Atwood, H.L., eds), pp. 241–292, Academic Press **[18] Glantz, R. M.** 1974. The visually evoked defense reflex of the crayfish: habituation, facilitation, and the influence of picrotoxin. *Journal of Neurobiology*, **5**, 263-280. **[19] Araki, M. & Nagayama, T.** 2005. Decrease in excitability of LG following habituation of the crayfish escape reaction. *Journal of Comparative Physiology A*, **191**, 481-489. **[20] Pereyra, P., González Portino, E. & Maldonado, H.** 2000. Long-Lasting and Context-Specific Freezing Preference Is Acquired after Spaced Repeated Presentations of a Danger Stimulus in the Crab *Chasmagnathus*. *Neurobiology of Learning and Memory*, **74**, 119-134. **[21] Rakitin, A., Tomsic, D. & Maldonado, H.** 1991. Habituation and sensitization to an electrical shock in the crab *Chasmagnathus*. Effect of background illumination. *Physiology & Behavior*, **50**, 477-487. **[22] Purdy, J. E., Dixon, D., Estrada, A., Peters, A., Riedlinger, E. & Suarez, R.** 2006. Prawn-in-a-tube procedure: Habituation or associative learning in cuttlefish? *The Journal of General Psychology*, **133**, 131-152. **[23] Long, T. M., Hanlon, R. T., Maat, A. T. & Pinsker, H. M.** 1989. Non-associative learning in the squid *Lolliguncula brevis* (Mollusca, Cephalopoda). *Marine & Freshwater Behaviour & Physiology*, **16**, 1-9.

Jumping spiders from the family Salticidae, also known as salticids, are well known for their orienting behaviour, or optomotor response. Salticid visual predatory behaviour typically consists of orientation, pursuit and capture (Forster 1982). The salticid orientation response is a whole body 'swivel' which has the goal of centring a moving stimulus, detected by one or more of the three pairs of secondary eyes, in a frontal fixation region of the field of view of the large primary (anterior medial, or AM) eyes for further inspection (Zurek and Nelson 2012a). Visual stimulation of the anterior lateral (AL) pair of secondary eyes alone can elicit an orientation response (Zurek and Nelson 2012b) and pursuit and capture predatory behaviour (Zurek *et al.* 2010). This suggests that the AL eyes have the ability to rapidly categorise stimuli and elicit the appropriate responses (Forster 1979; Zurek *et al.* 2010; Zurek and Nelson 2012b).

In the only study on salticids that has addressed habituation, albeit tangentially, Land (1971) found that when *Metaphidippus harfordi* and *M. aeneolus* experienced repetitive visual stimuli in the same region of the retina, they were faster at habituating than salticids that experienced stimuli in a new region of the retina. In order to further explore habituation in salticids, here I aimed to quantify whether, and to what extent, the orientation response of the salticid *Trite planiceps* coincides with the notion that the repeated application of a stimulus results in a progressive decrease in response to that stimulus, which is Thompson and Spencer's (1966) first characteristic of habituation. In particular, the purpose of the study was to determine the rate of habituation to a stimulus presented to a single AL eye compared to randomised stimuli presented to both AL eyes to determine whether stimulating different retinal areas (two eyes) would elicit a slower response decrement in a similar manner to that described by Land (1971).

Materials and Methods

Housing

Lighting in the lab was on a 12:12 L:D cycle commencing at 0800 hours. Temperature was kept constant at 25^{o} C. Adult *Trite planiceps* were housed in upside down transparent plastic jars (approximately 17 x 8 cm) with a cotton roll inserted in a drilled hole in the bottom of the lid which was partially submerged in a cup of water to ensure constant humidity. Two

additional holes were drilled into the opposite end of the jar; one covered with mesh for ventilation and the other plugged with a cork that could be removed for inserting houseflies (*Musca domestica*) or a *Drosophila* as food. All salticids were fed weekly on the day before experimental testing. Cages were cleaned after feeding.

Inside the jar was a folded piece of card in which *T. planiceps* could hide and build nests. As *T. planiceps* is typically found in the wild nesting alone in a single rolled leaf of flax (*Phormium tenax*), I inserted paper between the jars so that the *T. planiceps* could not see the other housed spiders.

Preliminary grid tests

For most of my experiments, identifying the fields of view of each pair of eyes was essential. *Trite planiceps* are larger than most salticids and may have a differently shaped cephalothorax to other species, possibly altering the fields of view of each pair of eyes from those identified for different species in the literature (e.g., Land 1985; Zurek 2010). Using a 1 cm^2 grid, I tested a total of six females to identify the field of view by covering specific pairs of eyes with Coltène® President dental silicon. Silicon was applied using a sharp steel probe under a stereomicroscope (Wild, 16x) to restrained salticids. For restraint, salticids were placed in a plastic plunger-like tube with one side filled with foam and the other side containing small holes from which the cephalothorax and eyes could be accessed. This permitted me to restrain the salticid in within without harm. The dental silicon blocks the view of the salticids and cannot be removed by the salticid itself. The dental silicon is removed cleanly without residue when pulled with tweezers and is an effective way of temporarily blinding the salticid without adverse effects.

First I tested two females with the posterior lateral (PL) and posterior medial (PM) secondary eyes covered as well as the primary anterior median (AM) eyes, in order to find the range of the anterior lateral (AL) eyes. Responses were counted when the salticid turned to look in the direction of a moving stimulus which was overlaid over a 1 x 1 cm grid across each monitor. This proved difficult, as they were not energetic and rarely oriented toward the stimulus because they were not pleased having their primary AM eyes covered. However, I was able to determine that the AL eyes were able to detect the stimulus as far as the midpoint of each the two monitors (Fig. 2.1). I then tested another two females by covering the PL and PM eyes to find the greatest distance from the centre of the screens that the AL eyes could see,

which proved to be 11 cm from the centre of the monitor. Lastly, I tested two females by covering only the AL eyes to illustrate the greatest distance from the centre of the two screens visible to the AM eyes and the range of the PL/PM eyes. This showed that the AM eyes could see a maximum of seven cm from the centre and that the PM/PL eyes could only see a range of 17-19 cm from the centre. Overall, this meant that to stimulate only the AL eyes the stimulus needed to appear solely within 8 - 16 cm (321 - 640 pixels) from the centre (midpoint) of the monitor (Fig. 2.1).

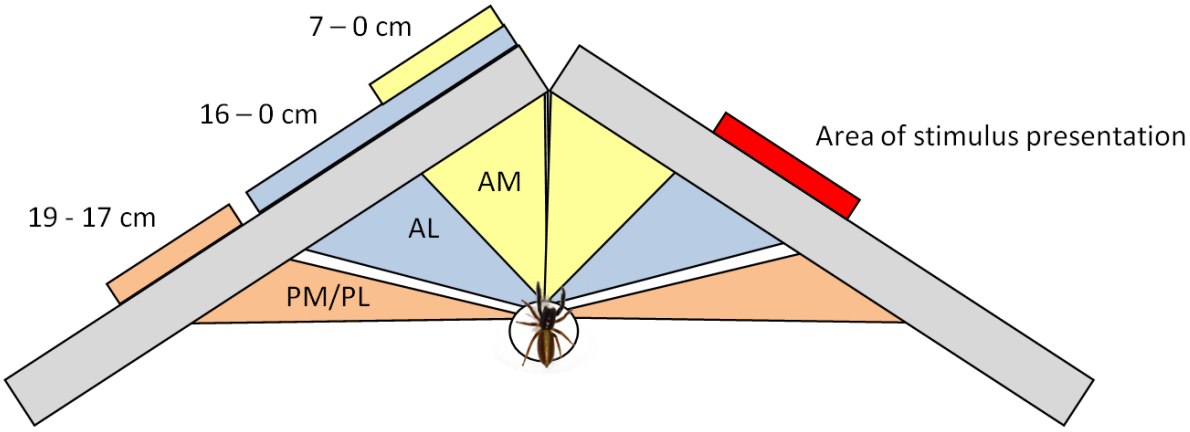

Fig. (2.1). Aerial view of the field of view of the salticid that elicited an orienting response for each eye pair, as determined by a stimulus displayed against grid display on each computer monitor (shaded grey). Eyes coded as: AM (anterior median), AL (anterior lateral), PL (posterior lateral) and PM (posterior median). Distances begin from the centre of one or other of each of the two monitors. Red area indicates where the stimulus was presented during testing.

Apparatus

The apparatus, or viewing rig, consisted of a holding stand, a camera, and two identical monitors (Fig. 2.2). The monitors were 17'' Phillips Brilliance 170P 1280 x 1042 with a refresh rate of 75 Hz. The monitors were standardised for brightness and colour using Spyder 4 Pro® colorimeter and accompanying software. The stimulus was a 40 pixel black circle (RGB 0,0,0) on a grey background (RGB 250, 250, 250) with a visual angle of 1.3˚ from the point of view of the test subject that moved across the screen between the range of 321 – 640 pixels from the centre to ensure its visibility was restricted to the AL eyes. The stimulus was designed to always cross on the horizon of the salticid's field of view (i.e., 0^{o} vertically) and moved from posterior to anterior. Stimulus presentation was controlled using a custom-written program using C# .NET on Microsoft Visual Studios ® 2010. This program enabled

control of stimulus type, size, vertical and horizontal location, velocity, duration, and interstimulus interval duration. Stimuli were created using Adobe Photoshop® CS5. All tests were video recorded remotely to avoid disturbing the salticid. Responses were recorded with a Logitech 1.3 Megapixel Rightlight™ Technology Webcam and corresponding Logitech software. Videos were used for subsequent behavioural analysis.

Testing protocol

Testing was carried out from 0830 to 1300 hours. Only female *T. planiceps* adults and sub-adults were used because male salticids are considerably less responsive toward stimuli than females and juveniles (Zurek *et al.* 2010). Salticids were used once per experiment and no salticid was tested more than three times overall.

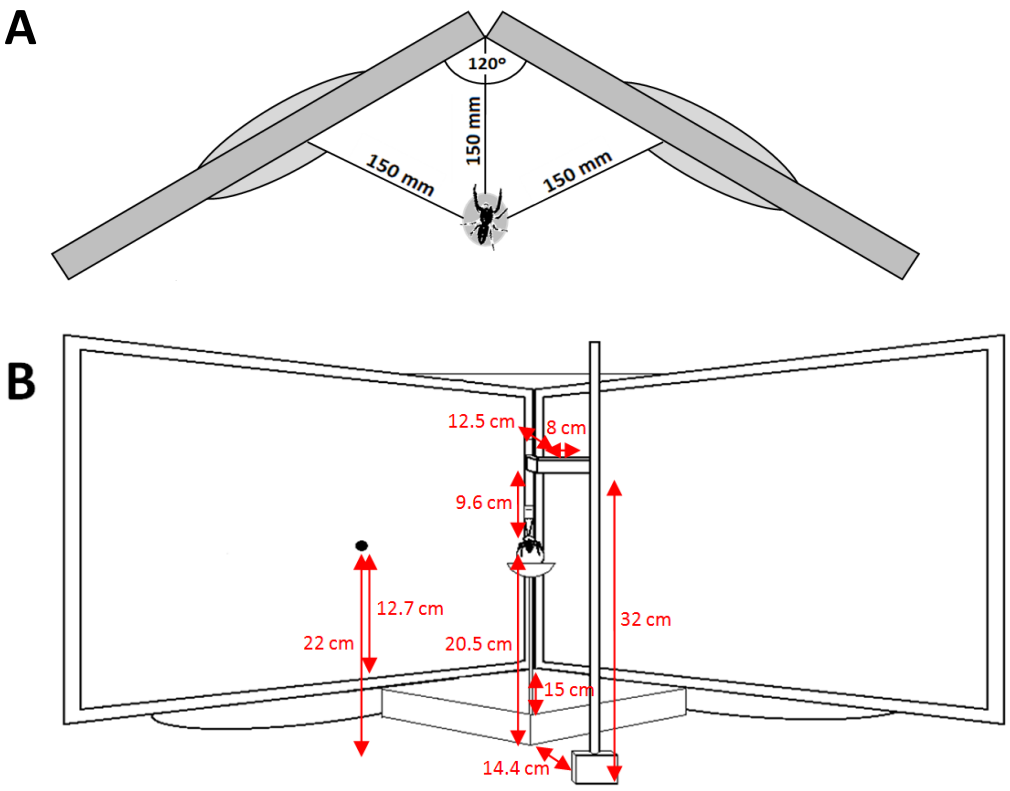

Fig. (2.2). Apparatus used for habituation tests of *Trite planiceps* salticids. (A) bird's-eye view of the position of the salticid on the polystyrene ball (shaded). A removable platform was used to ensure each salticid was the correct distance from the computer monitors (shaded). (B) Frontal view demonstrating the position of the rig holding the salticid and the cup underneath the ball for catching any sudden dismounts from the salticid. The dot shows the position of the stimulus in relation to the salticid.

Salticids were caught and placed in the plastic plunger for restraint. Once in position, wax was placed on the top of the salticid's cephalothorax to fix the TPC® disposable micro applicator termed the 'dental stick' (Fig 2.3). The dental stick with the salticid attached was then suspended within the rig by a crocodile clip. The salticid was then given a 15 mm crosshair marked polystyrene ball (weight 39 mg) to hold on to. This ball was cleaned with ethanol between test salticids to remove any chemicals deposited by previous individuals. Ten millimetres underneath the polystyrene ball was an elevated cup which served to catch the ball in case the salticid attempted to jump and dropped the ball. Once attached to the apparatus, the salticid had a full view of two identical computer monitors which were used to exhibited stimuli only within the view of the AL eyes. The salticid was suspended 150 mm from the computer screens (Fig. 2.2).

Fig. (2.3). A *Trite planiceps* female holds on to a marked polystyrene ball whilst being suspended from a dental stick attached with beeswax.

The purpose of the experiment was to determine if the rate of habituation to stimuli on a single side was faster than randomised stimuli presented on both monitors. I tested 20 salticids split into two groups of ten. Group A had the stimulus appear randomised on both monitors. Group B had the stimulus appear only on one monitor. Interstimulus interval was 20 seconds, with each stimulus appearing on screen for 5 seconds. The total number of stimulus presentations was 80 for both groups.

After being first attached to the apparatus the test salticid was given 20 minutes to settle. If the salticid dropped the ball and did not immediately pick it up the tests were halted for 10 minutes before continuing.

When presented with a stimulus, the salticid's orienting response causes the polystyrene ball that the salticid is holding to rapidly swivel in the opposite direction (Zurek and Nelson, 2012a). A rotation in any other direction or any other movement (e.g., very slow rotation or vertical movement of the ball) was not considered a response. I recorded the orientation response as a binomial variable, with orientation coded as 1 and failure to orient as 0. I also recorded any additional movement in any direction from the *T. planiceps* during the appearance of stimuli to expose any possible freeze response. Data were analysed using the software program R (2014). As the data were binary and each salticid was tested over many trials, I used a generalized linear mixed models (GLMM) with the family set as binomial, the

link function as Logit and set to the Laplace approximation (Waller *et al.* 2013). Data were averaged for graphing using a moving average of 5 trials and data were fitted with a non-linear exponential function.

Results

In the two conditions (both monitors random and one monitor), the orientation response rate to the repetitive visual stimuli rapidly declined during the first ten trials. After this initial period of rapid decline the salticids showed a slower decline in response rate, followed by an oscillating plateau in response rate over the remainder of the trials (Fig. 2.4), Nevertheless, there was a significant overall decrease in response over time for one monitor and both monitors (Table 2.1. comparison 1, 2). Salticids also habituated significantly more strongly when the stimulus appeared on a single monitor rather than randomised between both monitors (Table 2.1. comparison 3). The non-linear exponential function slope for a single monitor was Y = exp (-0.1804 (x+4.2825)) with the intercept as 0.225 and the slope for both monitors was Y = exp (-0.1786 (x+5.9474)) with an intercept of 0.158 (Fig. 2.4). There was a large amount of variation in response rate between individual salticids, as well as some form of oscillation in overall responses (Fig. 2.4).

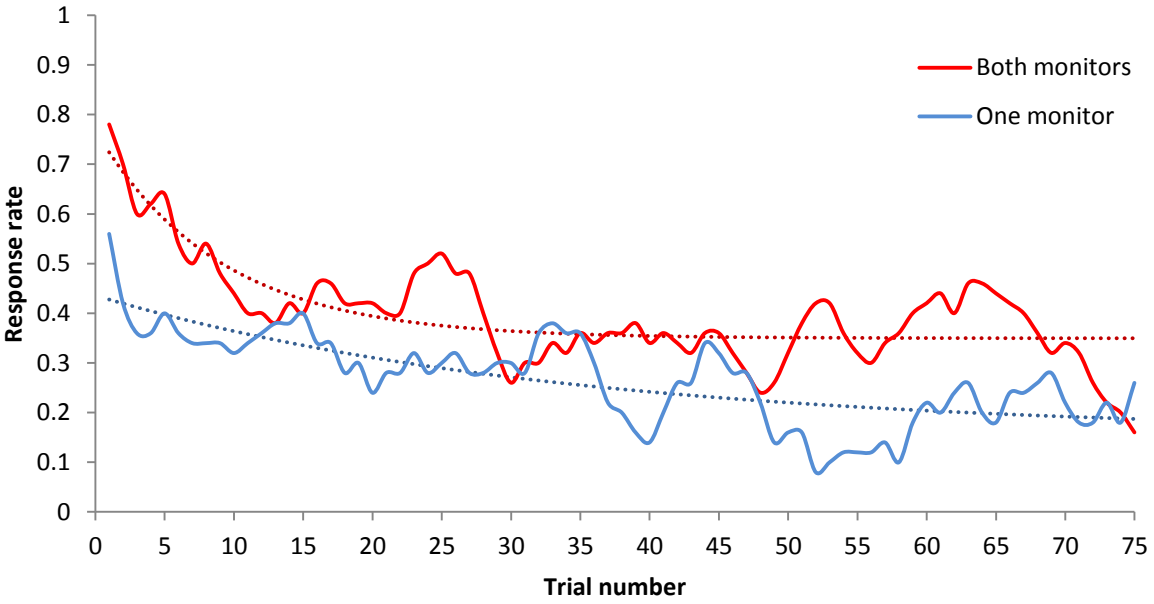

Fig. (2.4). Averaged orientation response data (solid lines), as proportion, fitted with a non-linear exponential function (dotted lines) in *Trite planiceps* over the number of trials. *T. planiceps* received stimuli from two computer monitors in one condition and only from one monitor in the other condition.

The graph depicted in Figure 2.4 and Figure 2.5 shows that with one monitor salticids began with a low response, at only about 55%, whereas with two monitors the response rate was nearer 80%. Although the Figure shows an initial response rate for the one sided condition of 55% this is an artefact of the smoothing action of the '5 trial moving mean' which averages the first five responses. In the one monitor condition most salticids stopped responding to the stimulus after only two trials, i.e. before five trials. When averaged, this gives the illusion that the first trial begun with a response rate of 55% but actually 100% of salticids responded to the first trial. This method of smoothing the data also results in the trial number ending at 75 instead of 80.

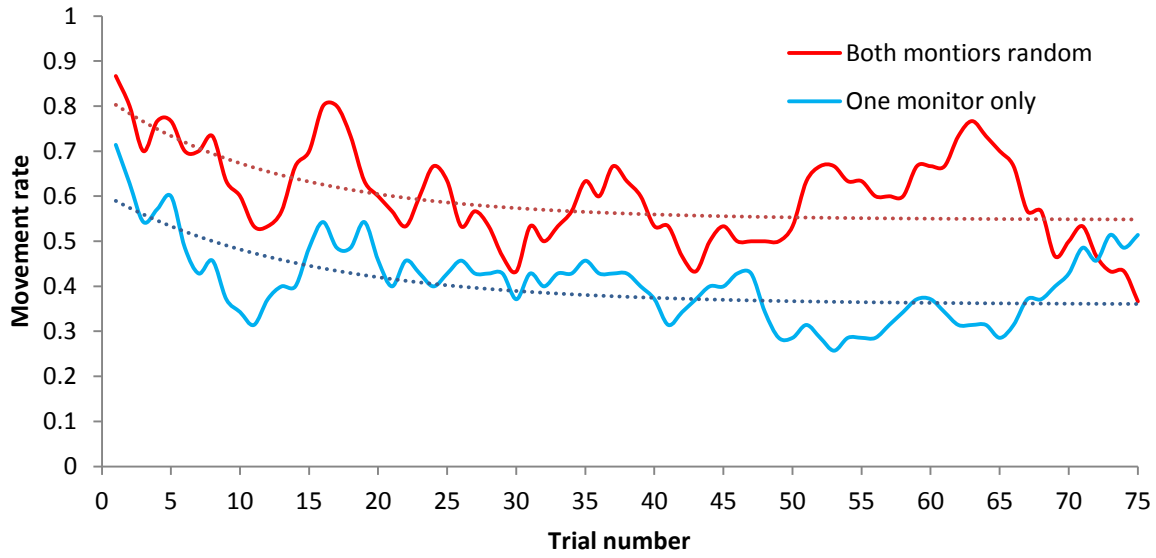

Fig. (2.5). Averaged data of all of *Trite planiceps* movement, as proportion, fitted with a non-linear exponential function. *T. planiceps* received stimuli from both of the computer monitors in one condition and only from one monitor in the other condition.

To investigate whether *T. planiceps* was performing a type of freeze response rather than habituation, I recorded all movements during testing. The salticids initially decreased and then maintained the same rate of movement throughout the trials within each of the two groups, indicating that they were not showing a freeze response, if this were the case we would expect the overall movement to rapidly drop close to zero, as opposed to the constant rate I observed (Fig 2.5) (Table 2.1. comparison 4). Overall movement from salticids in the condition with stimuli appearing on both monitors was higher than salticids which received the stimulus from one monitor only, mirroring results seen in Fig 2.4 (Fig. 2.5). This can also

be seen in the non-linear exponential function, as the slope of one screen equalled Y = exp (-0.0702 (x + 19.926)) with an intercept of 0.360 and the slope of both screens equalled Y = exp (-0.1.7121 (x + 7.5601)) with an intercept of 0.591 (Fig 2.5).

Table 2.1. GLMM results for habituation (H) and movement (F) experiments in which either orientation response or overall movement was recorded. See text for comparison details.

Experiment, comparison	**Test**	**Estimate**	**Standard error**	**z**	***p***	**N**
H, 1	Habituation response over trials one	-0.976	0.0978	-9.976	< 0.0001	10
H. 2	Habituation response over trials both	-0.413	0.0802	-5.149	< 0.0001	10
H, 3	Intercept one vs. both	-0.421	0.0883	-4.764	<0.0001	20
H, 3	One vs. both	-0.548	0.1095	-5.009	< 0.0001	20
F, 4	Intercept movement one vs. both	0.418	0.1001	4.183	<0.0001	13
F, 4	Movement one vs. both	-0.983	0.1361	-7.226	<0.0001	13

Discussion

Rather than show a slow progressive decline to near zero responses with repetitive stimulation, as is typical of the well-studied sea hare, *Aplysia californica* (Pinsker *et al.* 1970), the response rate of *T. planiceps* declined very rapidly within the first ten trials. This exceedingly rapid decline is consistent with Land's (1971) observations on other salticid species (*Metaphidippus harfordi* and *M. aeneolus*) responding to repeated visual stimuli. After this rapid decline *T. planiceps* exhibited a slower decline and then maintained roughly the same response rate, which oscillated between about 20 and 40%, over the rest of the trials and which never reached zero. This is similar to the findings made by Rakitin and colleagues (1991) on the crab *Chasmagnathus granulatus* with habituation to electric shocks (where presumably the stimulus was 'weak' enough to get a response decrement but 'strong' enough to maintain a level of watchfulness). It is possible that the size of our stimulus (40 pixels) is within the expected size range of a distant salticid which could be a potential predator or mate (hence the maintained level of responsiveness), although this seems unlikely because the size

of the stimulus has a visual angle of only 1.3˚ from the point of view of the salticid, and as such this source would be at some considerable distance from the test subject. Nevertheless, it is conceivable that a smaller stimulus would see the response decrement reach zero, or near zero.

The salticids showed a lower response rate when the stimulus appeared only on one side compared to appearing randomly on both sides. These findings also coincide with Land's (1971) work in which he found that the salticids habituated faster when they experienced a stimulus within the same visual field compared to salticids that experienced a stimulus in a new region. Land (1971) mentioned this finding from the view point that the more rapid habituation was mainly due to the same region of the retina being stimulated, and this may well be the case with *T. planiceps,* particularly when only one monitor was used as ISI was constant, whereas when the stimulus was randomised between monitors ISI varied depending on the randomisation (i.e., it could be 20 seconds or over a minute between consecutive trials in the same region of the same eye).

One potential confounding factor in habituation testing is the animal switching from orienting behaviour to freeze behaviour, which may appear to be habituation due to the lack of response to the stimulus. However, an important distinction should be made between an animal employing a motionless type of anti-predator behaviour and habituation. During habituation trials of a shadow stimulus imitating a predator moved overhead, Pereyra and colleagues (2000) countered this issue by recording the body positions of the crab (*Chasmagnathus granulatus*) as freezing, resting, escaping, and wandering. They found that the crabs would show freezing behaviour when the experiments were of a short duration with breaks in between or had long ISI (171 seconds), but would not show this freeze behaviour when the experiments were long or had an ISI of 0 seconds. This means that the frequency and/or the duration of the stimuli were determining factors in whether the crabs habituated or employed a freeze response (Pereyra *et al.* 2000). With *T. planiceps* I recorded all other movement from the salticid during habituation testing. That the salticids maintained the same rate of movement throughout the trials suggests that they were not showing a freeze response (as we would expect the overall movement to drop to zero if this were the case). Interestingly, the overall movement from salticids in the condition with stimuli appearing on both monitors was higher than salticids who received the stimulus from one monitor only, which reflects the results from tests of habituation of orienting responses. This shows that the orientation

response and overall movement was affected by whether stimuli appeared on one monitor or both monitors.

To summarise, *T. planiceps* exhibited a response decrement similar to habituation and showed a higher response rate to stimuli appearing randomly on two monitors than to stimuli only appearing on one. This difference in response rate for the stimulus appearing on both screens could be (1) by being randomised between monitors the stimuli are more unpredictable so potentially more threatening, leading to raised awareness, (2) because each eye out of the two was only stimulated half the number of times when presented on both monitors or (3) - and related to (2) - that the ISI between stimulation of the specific eye receiving input could vary depending on the condition (if presented on a single monitor ISI was constant, but when randomly presented on two monitors there could be considerable variation in the interval in which the same eye was stimulated again). The latter two are potentially flaws that make interpretation of these data difficult, and further experiments (see Chapter 3) were designed to rectify these issues.

References

Artigas, A. A., Sansa, J. & Prados, J. 2012. Distractor effects upon habituation of complex stimuli. *Behavioural Processes*, **90**, 204-209.

Barry, R. J. 2009. Habituation of the orienting reflex and the development of Preliminary Process Theory. *Neurobiology of Learning and Memory*, **92**, 235-242.

Boulis, N. M. & Sahley, C. 1988. A behavioral analysis of habituation and sensitization of shortening in the semi-intact leech. *The Journal of Neuroscience*, **8**, 4621-4627.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics*, **77**, 71-94.

Christensen, J. W., Zharkikh, T. & Chovaux, E. 2011. Object recognition and generalisation during habituation in horses. *Applied Animal Behaviour Science*, **129**, 83-91.

Dong, S. & Clayton, D. F. 2009. Habituation in songbirds. *Neurobiology of Learning and Memory*, **92**, 183-188.

Dukas, R. & Clark, C. W. 1995. Sustained vigilance and animal performance. *Animal Behaviour*, **49**, 1259-1267.

Edwards, D. H., Heitler, W. J. & Krasne, F. B. 1999. Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. *Trends in Neurosciences*, **22**, 153-161.

Engel, J. E. & Wu, C. F. 2009. Neurogenetic approaches to habituation and dishabituation in *Drosophila*. *Neurobiology of Learning and Memory*, **92**, 166-175.

Fennell, C. T. 2012. *Research Methods in Child Language: A Practical Guide*. Habituation Procedures. 1-16.

Gee, W. 1913. The behavior of leeches with especial reference to its modifiability. *University of California Publications in Zoology*. **11**, 197-305.

Giles, A. C. & Rankin, C. H. 2009. Behavioral and genetic characterization of habituation using *Caenorhabditis elegans*. *Neurobiology of Learning and Memory*, **92**, 139-146.

Gover, T. D. & Abrams, T. W. 2009. Insights into a molecular switch that gates sensory neuron synapses during habituation in *Aplysia*. *Neurobiology of Learning and Memory*, **92**, 155-165.

Groves, P. M. & Thompson, R. F. 1970. Habituation: a dual-process theory. *Psychological Review*, **77**, 419.

Hawkins, R. D., Kandel, E. R. & Bailey, C. H. 2006. Molecular mechanisms of memory storage in *Aplysia. The Biological Bulletin*, **210**, 174-191.

Klingner, C. M., Hasler, C., Brodoehl, S. & Witte, O. W. 2014. Excitatory and inhibitory mechanisms underlying somatosensory habituation. *Human Brain Mapping*, **35**, 152-160.

Krasne, F. B. 1969. Excitation and habituation of the crayfish escape reflex: the depolarizing response in lateral giant fibres of the isolated abdomen. *Journal of Experimental Biology*, **50**, 29-46.

Krasne, F.B. & Teshiba, T.M. 1995. Habituation of an invertebrate escape reflex due to modulation by higher centers rather than local events. *Proceedings of the National Academy of Sciences*. U. S. A., **92**, 3362–3366.

Kupfermann, 1., Carew, T. J. and Kandel, E. R. 1974. Local, reflex, and central commands controlling gill and siphon movements in Aplysia. *Journal of Neurophysiology*. **37**, 996-1019.

Land, M. 1971. Orientation by jumping spiders in the absence of visual feedback. *Journal of Experimental Biology*, **54**, 119-139.

Land, M. 1985. The morphology and optics of spider eyes. In: *Neurobiology of arachnids*, pp. 53-78: Springer Berlin Heidelberg.

Mackworth, J. F. 1968. Vigilance, arousal, and habituation. *Psychological Review*, **75**, 308.

Martinez, J. L., & Kesner, R.P. 1998. *Neurobiology of Learning and Memory*. San Diego.: Academic Press Inc.

Mazur, J. E. 2006. *Learning and Behavior*, Sixth edition. Upper Saddle River, NJ.: Pearson Prentice Hall.

Mittenthal, J. E. & Wine, J. J. 1973. Connectivity patterns of crayfish giant interneurons: visualization of synaptic regions with cobalt dye. *Science*, **179**, 182-184.

McSweeney, F. K. & Murphy, E. S. 2009. Sensitization and habituation regulate reinforcer effectiveness. *Neurobiology of Learning and Memory*, **92**, 189-198.

Pinsker, H., Kupfermann, I., Castellucci, V. & Kandel, E. 1970. Habituation and dishabituation of the GM-withdrawal reflex in *Aplysia*. *Science*, **167**, 1740-1742.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rakitin, A., Tomsic, D. & Maldonado, H. 1991. Habituation and sensitization to an electrical shock in the crab *Chasmagnathus*. Effect of background illumination. *Physiology & Behavior*, **50**, 477-487.

Ramaswami, M. 2014. Network Plasticity in Adaptive Filtering and Behavioral Habituation. *Neuron*, **82**, 1216-1229.

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C.-F. & Thompson, R. F. 2009. Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. *Neurobiology of Learning and Memory*, **92**, 135-138.

Rankin, C. H., Beck, C. D. O. & Chiba, C. M. 1990. *Caenorhabditis elegans*: A new model system for the study of learning and memory. *Behavioural Brain Research*, **37**, 89-92.

Rankin, C. H. & Carew, T. J. 1987. Development of learning and memory in Aplysia. II. Habituation and dishabituation. *The Journal of neuroscience*, **7**, 133-143.

Rose, J. K. & Rankin, C. H. 2001. Analyses of habituation in *Caenorhabditis elegans*. *Learning & Memory*, **8**, 63-69.

Schloegl, C., Kotrschal, K. & Bugnyar, T. 2007. Gaze following in common ravens, *Corvus corax*: ontogeny and habituation. *Animal Behaviour*, **74**, 769-778.

Sharpless, S. & Jasper, H. 1956. Habituation of the arousal reaction. *Brain*, **79**, 655-680.

Stiles, B. W. & Ghosh, J. 1997. Habituation based neural networks for spatio-temporal classification. *Neurocomputing*, **15**, 273-307.

Thompson, R. F. 2009. Habituation: A history. *Neurobiology of Learning and Memory*, **92**, 127-134.

Thompson, R. F. & Spencer, W. A. 1966. Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychological Review*, **73**, 16.

Waller, B., Warmelink, L., Liebal, K., Micheletta, J. & Slocombe, K. 2013. Pseudoreplication: a widespread problem in primate communication research. *Animal Behaviour*, **86**, 483-488.

Wood, D. C. 1988. Habituation in *Stentor*: produced by mechanoreceptor channel modification. *The Journal of Neuroscience*, **8**, 2254-2258.

White, J. G., Southgate, E., Thomson, J. N. & Brenner, S. 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, **314**, 1-340.

Wine, J.J. & Krasne, F.B. 1982. The Biology of Crustacea Neural Integration and Behaviour (Vol. 4) (Sandeman, D.C. and Atwood, H.L., eds), 241–292, Academic Press.

Zelazo, P. R., Brody, L. R. & Chaika, H. 1984. Neonatal habituation and dishabituation of head turning to rattle sounds. *Infant Behavior and Development*, **7**, 311-321.

Zucker, R.S. 1972. Crayfish escape behavior and central synapses: I. Neural circuit exciting lateral giant fiber. *Journal of Neurophysiology*. **35**, 638–651.

Zurek, D. B., Taylor, A. J., Evans, C. S. & Nelson, X. J. 2010. The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *Journal of Experimental Biology*, **213**, 2372-2378.

Zurek, D. B. & Nelson, X. J. 2012a. Hyperacute motion detection by the lateral eyes of jumping spiders. *Vision Research*, **66**, 26-30.

Zurek, D. B. & Nelson, X. J. 2012b. Saccadic tracking of targets mediated by the anterior-lateral eyes of jumping spiders. *Journal of Comparative Physiology A*, **198**, 411-417.

Chapter 3.

Life in the fast lane: does more rapid stimulation result in quicker habituation with jumping spiders?

Habituation is the decrease in the strength (including complete cessation) of a response after repeated presentation of a stimulus which elicits the response (Mackworth 1968; Mazur 2006). By enabling an animal to distinguish biologically 'irrelevant' background stimuli from biologically important stimuli, this process protects the organism's nervous system from being flooded with irrelevant information (Klingner *et al.* 2014). Habituation is a separate process to sensory adaptation or fatigue (Rankin *et al.* 2009). Fatigue is defined as the failure to maintain the required or expected power output, usually seen as a decrease in force and muscle action potential as stimulation progresses (Cooper *et al.* 1988). Additionally, response decrements resulting from very rapid stimulation (e.g. 500 Hz), trauma, and growth or aging are not classified as habituation (Thompson and Spencer 1966). Habituation can be identified from other response decrements because it is reversible, either after a rest break (spontaneous recovery) or through the presentation of a novel stimulus (dishabituation). Habituation does not require conscious motivation or awareness (Klingner *et al.* 2014) and has been demonstrated (at least to some extent) in most animals, from the protozoan *Stentor* (Wood 1988) to humans (Geer 1966). However, of the ten traits defined as characteristics of habituation (see Thompson and Spencer 1966; Groves and Thompson 1970; Rankin *et al.* 2009), very few animals have been tested across the full spectrum. Here I aim to explore in depth one of the characteristics proposed, which is that more frequent stimulation results in a faster or more pronounced response decrement.

The phenomenon that interstimulus interval (ISI) can affect the strength of habituation has been investigated in several invertebrates. *Caenorhabditis elegans* responds to the mechanical stimulus of a tap to the Petri dish in which the nematode is housed by rapidly swimming backwards. Rankin and colleagues explored habituation of the tap withdrawal response and found that the ISI determined the rate of habituation in *C. elgans* (Rose and Rankin 2001; Giles and Rankin 2009). When compared to an ISI of 60 seconds, an ISI of 10 seconds created a faster decrement in loss of response (rapid habituation), as well as causing

a lower maintained response rate over the rest of the trials once habituated (Rose and Rankin 2001).

Using a different approach, Araki and Nagayama (2005) explored ISI in the crayfish *Procambarus clarkia.* Here, the authors isolated the lateral giant abdominal nerve cord and used electrodes to stimulate the neurons in pulses using different ISI times. Interestingly, Araki and Nagayama (2005) found that with an ISI of one second the response dropped by 80% after only four trials, with an ISI of five seconds causing an 80% decline after 20 trials. However, an ISI of 20 or 60 seconds caused a drop of only 50% responses after 20 trials, demonstrating that the difference in response decrement between interstimulus intervals of one and five seconds ISI was greater than the response decrement to the longer intervals of 20 to 60 seconds (Araki and Nagayama 2005). These results again support the notion that more frequent stimulation exacerbates habituation.

Aplysia californica has been a foundation model animal for habituation research due to the simplicity of the neural network and the ease with which individual neurons can be investigated due to their large size (Glanzman 2009). The defensive withdrawal reflexes, such as the readily observable gill-withdrawal reflex, habituate to repeated mechanical stimulation on the body as well as to intracellular stimulation of the sensory pathway (Pinsker *et al.* 1970). When testing habituation of the gill-withdrawal reflex along the sensory pathway, Byrne (1982) found that an ISI of one second caused a rapid decrease of the median amplitude of the excitatory postsynaptic potential (EPSP) (< 50% amplitude response) by the third stimulus. However, an ISI of 100 seconds only caused a decline of the median EPSP amplitude to 75% of the non-habituated response, even by the tenth stimulus. In contrast, interstimulus intervals of 3, 10 and 30 seconds showed little differences in median EPSP amplitude (just over 50%) by the tenth stimulus (Byrne 1982). This shows a clear difference in habituated responses with intervals of one and 100 seconds, the ends of the two extremes, but not a great deal of difference in response for intermediate interval times, although the pattern of longer interstimulus intervals requiring more repetitions in order to elicit a response decrement is evident.

Research on leeches (*Hirudo medicinalis*), however, suggests a different trend, as habituation rate with an ISI of 44 seconds was slower than one of 300 seconds, yet no habituation at all was found with an ISI of ten seconds (Boulis and Sahley 1988). These somewhat paradoxical

results may have been influenced by the leech being set up as a semi-intact preparation in which the animal was transected at the ninth segment, exposing two ganglia and the associated sinus.

Overall, the body of work on the effect of ISI on habituation rate in invertebrates shows that there are differences depending on species, or possibly depending on the testing protocols used. Here I use behavioural assays to test the effect of ISI for the first time on a salticid, *Trite planiceps*. My predictions are: 1. That shorter interstimulus intervals between visual stimuli will cause a more rapid decline in response. 2. That shorter interstimulus intervals will result in a more enduring low response rate than longer intervals. In the experiment described in Chapter 2 I encountered a methodological concern because salticids in one condition potentially received half of the number of stimuli per eye compared with the other condition. To remedy this potential problem, in this study I presented visual stimuli such that each eye received the same number of stimuli in an alternating pattern for all conditions.

Materials and Methods

Methods were identical to those described in Chapter 2 apart from specific details outlined below. Ten salticids, divided into two groups of five, were tested with a visual stimulus of a black circle subtending 1.3˚ from the point of view of the test subject which appeared on both monitors in an alternating fashion. Group 1 had an ISI of 20 seconds, while group 2 had an ISI of ten seconds. For this experiment the total number of repetitions was increased from the 80 used in Chapter 2 to 160. This changed the total test duration from 40 minutes to 80 minutes. By doubling the repetitions of the stimulus and changing the order of stimulus presentation appearing on two monitors from random to alternating, I eliminated the possibility that each eye received only half or an unequal number of stimulus repetitions, which was a problem in Chapter 2. As in Chapter 2, video recorded responses were scored as a binomial variable, where orientation toward the stimulus (causing the polystyrene ball held by the salticid to rapidly swivel in the opposite direction from the stimulus) was scored as 1. A rotation in any other direction, or any other movement, was not considered a response and was scored as 0. Data were analysed using R (2014). As each salticid underwent 160 trials, I used generalized linear mixed models (GLMM) with random effects. The family was set as

binomial, and the link function was set as Logit and set to a Laplace approximation (Waller *et al.* 2013). Data were averaged for graphing using a moving average of 5 trials and I fitted a non-linear exponential function to these data. I also compared the data using ISI of 20 s from Chapter 2 (randomised presentation) to those obtained here with 20 s ISI (alternating presentation) to investigate the effects of constant stimulation in a single eye, as opposed to two eyes with the same ISI.

Results

In the two conditions (10 and 20 s ISI), the orientation response rate of *T. planiceps* to repetitive visual stimuli rapidly declined in the first fifteen trials (Fig 3.1). After this rapid decline the salticids showed a slower decline and then maintained an oscillating lowered response rate over the remainder of the trials, irrespective of condition (Table 3.1 comparison 1, 2). The non-linear exponential function fitted to the averages from each condition shows that the 10 s ISI declines more rapidly than 20 s ISI, with a lower response rate overall being maintained.

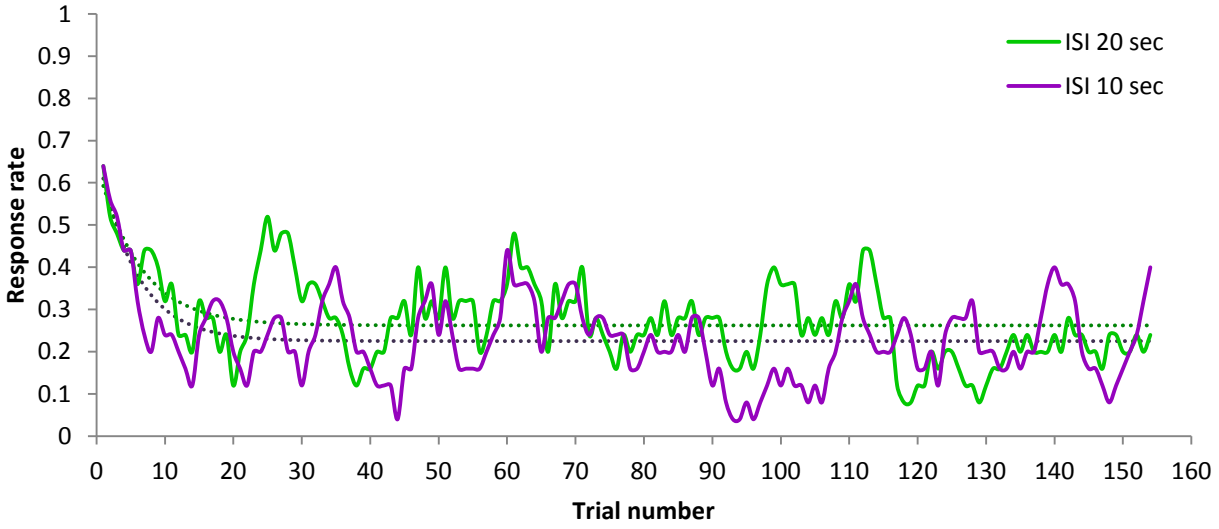

Fig. (3.1). Five moving mean smoothed averaged data (solid lines) of *Trite planiceps* response rate to a visual stimulus with different interstimulus intervals (10 s or 20 s). Data fitted with a non-linear exponential function (dotted lines).

The non-linear exponential function slope for 10 s ISI was Y= exp (-0.2979 (x+1.662385)) with an intercept of 0.2241 and the slope for 20 s ISI was Y = exp (-0.1804 (x+4.2824)) with an intercept of 0.2249 (Fig. 3.1). GLMMs showed that response rate decreased significantly as trials progressed for both the 10 s ISI, and 20 s ISI conditions (Table 3.1 comparison 1, 2).

The decrease in response over trials was related to ISI, with shorter ISI producing a significantly stronger habituated response (Table 3.1. comparison 3).

I was able to compare the orientation response rate of *T. planiceps* to stimuli appearing on one monitor, two monitors randomly, and two monitors alternating by combining the 20 s ISI data from Chapter 2 to those obtained here. A non-linear exponential function was fitted to each of the averaged data, with the slope of random presentation of the stimulus on the two monitors being Y = exp (-0.1786 (x + 5.9474)) with an intercept of 0.1577. The equations describing the fit for stimulus presentation on a single monitor and two alternating monitors were very similar, with the slope of one monitor being Y = exp (-0.1804 (x + 4.2825)) with an intercept of 0.2249, and two alternating monitors being Y = exp (-0.1804 (x + 4.2824)) with an intercept of 0.2249. Indeed, it is evident from Figure 3.2 that the conditions 'one monitor only' and 'both monitors alternating' appear closer together, and lower, in response rate values than 'both monitors alternating', with the main difference between the former two being the sharper decline in response with both monitors presenting the stimulus in alternating fashion. However, when the stimulus is presented in alternating fashion, there does appear to be an overall higher habituated response (of around 30%), which is maintained. In contrast, with presentations on a single monitor, the response continues to decline with increasing number of trials. Additionally, when presentations are randomised, the maintained overall response is considerably higher (around 40%) than when stimulus presentation is constant, either on one or two monitors.

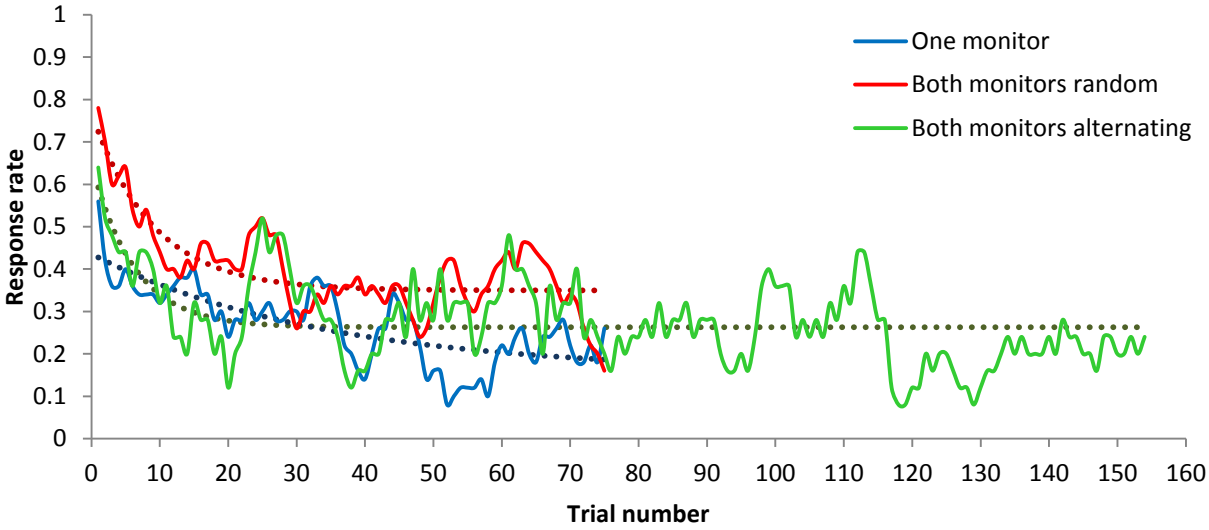

Fig. (3.2). Smoothed averaged data (solid lines) fitted with a non-linear exponential function (dotted lines) from Chapter 2 and Chapter 3 of response decrement in *Trite planiceps* when stimuli appear on either one monitor (20 s ISI), two monitors randomly (ISI variable but average 20 s ISI) or two monitors alternating (20 s ISI).

To recap from the previous chapter, there was a significant overall decrease in response rate to repeated stimulation over the trials for one monitor, both monitors randomly and both monitors alternating (Table 3.1 comparison 4-6). With the combined data from Chapter 2 and this chapter, I was able to confirm that testing condition (stimulus presentation on one monitor, both monitors randomly, or both monitors alternating) significantly affected response decrement (Table 3.1 comparison 7).

Table 3.1. GLMM results for interstimulus interval (ISI) and habituation (H) experiments. See text for comparison details.

Experiment, comparison	**Test**	**Estimate**	**Standard error**	**z**	***p***	**N**
ISI, 1	10 ISI responses decreased over trials	-1.0701	0.153	-6.98	< 0.0001	5
ISI, 2	20 ISI responses decreased over trials	-0.720	0.125	-5.766	< 0.0001	5
ISI, 3	Intercept 10 vs. 20 s ISI	-1.190	0.0973	-12.259	<0.0001	20
ISI, 3	10 vs. 20 s ISI	0.241	0.111	2.026	0.043	20
H, 4	One monitor decreased over trials	-0.976	0.0978	-9.976	< 0.0001	10
H, 5	Both random decreased over trials	-0.413	0.0802	-5.149	<0.0001	10
H, 6	Both alternating decreased over trials	-0.720	0.125	-5.766	< 0.0001	10
H, 7	Intercept one vs. both random vs. both alternating	-0.421	0.0883	-4.764	<0.0001	30
H, 7	One vs. both random vs. both alternating	-0.548	0.109	-5.009	< 0.0001	30

Discussion

This is the first study to examine the effect of interstimulus interval on response decrement in salticids. My results show that the ISI had a significant effect in the initial response decrement of *Trite planiceps* toward visual stimuli, which was then maintained throughout

the remaining trials. While it seems possible that a larger sample size and greater difference in ISI (such as 10 seconds versus 60 seconds, rather than 10 and 20 seconds as tested here) would reduce variability and other sources of 'noise' and more clearly illustrate the effect of ISI, the effects were clearly visible despite the small sample size.

These tests were designed to control for the possibility of fatigue, defined as the failure to maintain the required or expected power output (Cooper *et al.* 1988), affecting the results. After 160 trials the overall level of orienting responses, which requires considerable motor coordination for eights legs to rapidly 'swivel' toward the source of motion, was still higher than it was with half the number of presentations with the same ISI (Chapter 2), seemingly ruling out fatigue as a factor in these tests. If physical fatigue were to become apparent the response decrement would continue to decline over time. This would become especially obvious when comparing 160 trials, taking place over 80 minutes, with 80 trials taking place over 40 minutes. Additionally, the salticids did not show a gradual decline, as is typical of fatigue (Cooper *et al.* 1988); instead, the salticids quickly decreased their response rate and then maintained the same oscillating baseline throughout the remaining trials. However, to be able to rule out fatigue entirely I would need to comply with Thompson and Spencer's (1966) guidelines that habituation can be reversed by showing recovery of the response either after a rest break (spontaneous recovery) or through the presentation of a novel stimulus (dishabituation).

Pereyra and collegues (2000) discovered a difference in behaviours depending on the ISI and duration of habituation experiments with the crab *Chasmagnathus granulata*. During habituation trials with a stimulus shadow moving overhead imitating a predator, they recorded the body positions of the crab as either freezing, resting, escaping, or wandering. They found that the crabs would exhibit freezing behaviour when the experiments were of a short duration or had an ISI of 171 seconds, but when the experiments were of a long duration or had an ISI of 0 seconds the crabs would habituate to the overhead shadow. This means that both the stimulus frequency and the duration of the experiments were determining factors in whether the crabs habituated to the stimuli or employed a freeze response (Pereyra *et al.* 2000). In Chapter 2 I recorded overall movement during testing of *Trite planiceps* and found that the salticids did not employ a freeze response. But, these results along with the ones previously mentioned with the mollusc *Aplysia californica* (Byrne 1982), the nematode *Caenorhabditis elegans* (Rose and Rankin 2001), and the crayfish *Procambarus clarkia*

(Araki and Nagayama 2005), indicate that there is a range of interstimulus intervals where the animal is more responsive to either very short or very long interval durations compared with mid range interval durations. This suggests the possibility that there may be two different mechanisms for habituation; a 'short term' and a 'long term' process. Although beyond the scope of this study, research on *A. californica* suggests cellular mechanisms whereby this might occur (see Glanzman 2009). While working directly with the nervous system of salticids is currently intractable, it would be interesting to use behaviour to explore short-term and longer-term processes further in *Trite planiceps* to see if a similar pattern in responsiveness to ISI values is found.

Another characteristic of habituation described by Thompson and Spencer (1966) was the appearance of spontaneous recovery of the habituated response when given a break from habituation testing. Although I didn't explore this characteristic, Rose and Rankin (2001) tested whether *C. elegans* altered their spontaneous recovery when subjected to tests using different ISI, finding that ISI altered the rate of spontaneous recovery. *C. elegans* which had been given short ISI displayed rapid spontaneous recovery while those given longer ISI showed much slower spontaneous recovery (Rose and Rankin 2001). Rose and Rankin (2001) suggest that this method for testing habituation is a reliable indicator of habituation, as it has the opposite recovery response to fatigue. This method of testing for spontaneous recovery with different ISI would be another interesting procedure to test on *Trite planiceps* to confirm whether the response decrement seen here and in Chapter 2 was truly habituation.

I compared the response rates between stimuli appearing on one monitor, both monitors alternating and both monitors randomly to investigate the hypothesis that receiving 'half the number of stimuli per eye' (random presentations) was causal to a higher maintained response rate. This comparison yielded interesting results, as salticids given random presentations of the stimulus exhibited higher response rates (c. 40% maintained over time) than either stimulus presentation on one monitor (c. 25%, and continuing to decline with time) or on alternating monitors (c. 30% maintained over time), even taking into account different test durations and the number of stimulus presentations. This suggests that the 'half the number of stimuli per eye' hypothesis was not the sole reason for a higher response rate because we would also expect to see higher response rates in the alternating condition. Although the alternating condition did have a slightly higher overall maintained response compared with single monitor presentations, these were considerably lower than those seen

with salticids in the random presentation treatment. The results presented here suggest that, due to the random nature of stimulus presentation, the salticids maintained a higher response rate in this condition because stimulation was not as predictable as in the other two conditions. To corroborate this hypothesis I would need to re-test the salticids using 160 trials with stimuli appearing randomly on both screens.

While further testing is certainly required to rule out all other possibilities, overall my results strongly suggest that *Trite planiceps* habituates to repeated presentation of a visual stimulus, and that the response decrement is not driven by fatigue, but instead by ISI, with faster stimulus presentation leading to a more pronounced, and sustained, loss of response.

References

Araki, M. & Nagayama, T. 2005. Decrease in excitability of LG following habituation of the crayfish escape reaction. *Journal of Comparative Physiology A*, **191**, 481-489.

Boulis, N. M. & Sahley, C. 1988. A behavioural analysis of habituation and sensitization of shortening in the semi-intact leech. *The Journal of Neuroscience*, **8**, 4621-4627.

Byrne, J. H. 1982. Analysis of synaptic depression contributing to habituation of gill-withdrawal reflex in *Aplysia californica*. *Journal of Neurophysiology*, **48**, 431-438.

Cooper, R., Edwards, R., Gibson, H. & Stokes, M. 1988. Human muscle fatigue: frequency dependence of excitation and force generation. *The Journal of Physiology*, **397**, 585-599.

Geer, J. H. 1966. Effect of interstimulus intervals and rest-period length upon habituation of the Orienting response. *Journal of Experimental Psychology*, **72**, 617.

Giles, A. C. & Rankin, C. H. 2009. Behavioural and genetic characterization of habituation using *Caenorhabditis elegans*. *Neurobiology of Learning and Memory*, **92**, 139-146.

Glanzman, D. L. 2009. Habituation in *Aplysia*: the Cheshire cat of neurobiology. *Neurobiology of Learning and Memory*, **92**, 147-154.

Groves, P. M. & Thompson, R. F. 1970. Habituation: a dual-process theory. *Psychological Review*, **77**, 419.

Klingner, C. M., Hasler, C., Brodoehl, S. & Witte, O. W. 2014. Excitatory and inhibitory mechanisms underlying somatosensory habituation. *Human Brain Mapping*, **35**, 152-160.

Mackworth, J. F. 1968. Vigilance, arousal, and habituation. *Psychological Review*, **75**, 308.

Mazur, J. E. (2006). *Learning and Behaviour*, Sixth edition. Upper Saddle River, NJ.: Pearson Prentice Hall.

Pereyra, P., González Portino, E. & Maldonado, H. 2000. Long-Lasting and Context-Specific Freezing Preference Is Acquired after Spaced Repeated Presentations of a Danger Stimulus in the Crab *Chasmagnathus*. *Neurobiology of Learning and Memory*, **74**, 119-134.

Pinsker, H., Kupfermann, I., Castellucci, V. & Kandel, E. 1970. Habituation and dishabituation of the GM-withdrawal reflex in *Aplysia*. *Science*, **167**, 1740-1742.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C.-F. & Thompson, R. F. 2009. Habituation revisited: An updated and revised description of the behavioural characteristics of habituation. *Neurobiology of Learning and Memory*, **92**, 135-138.

Rose, J. K. & Rankin, C. H. 2001. Analyses of habituation in *Caenorhabditis elegans*. *Learning & Memory*, **8**, 63-69.

Thompson, R. F. & Spencer, W. A. 1966. Habituation: a model phenomenon for the study of neuronal substrates of behaviour. *Psychological Review*, **73**, 16.

Waller, B., Warmelink, L., Liebal, K., Micheletta, J. & Slocombe, K. 2013. Pseudoreplication: a widespread problem in primate communication research. *Animal Behaviour*, **86**, 483-488.

Wood, D. C. 1988. Habituation in *Stentor*: produced by mechanoreceptor channel modification. *The Journal of Neuroscience*, **8**, 2254-2258.

Chapter 4.

Shock to the system: do jumping spiders dishabituate?

Animals that experience repeated stimulation with the same stimulus often show a habituated, or decreased, response to the stimulus after a period of time (Mackworth 1968; Mazur 2006). However, it is now well established that a habituated animal can also become 'dishabituated' and resume normal responses in certain circumstances, such as when given a novel stimulus. Holmes (1912) first used the terms "dishabituation" and "dehabituation" in his work on the sea urchin *Arbacia punctulata*. Pavlov (1927) described the process of dishabituation in relation to classical conditioning and the habituated orienting response (cited in Thompson and Spencer 1966). Dishabituation was seen as the neutralisation of habituation (Humphrey 1933), then a form of sensitisation by (Groves and Thompson 1970), and is currently seen as a method to ensure that true habituation is taking place, instead of another response decrement such as physical fatigue (Thompson and Spencer 1966). However, the exact mechanism of dishabituation and its relationship to habituation remains somewhat mysterious (Rankin *et al.* 2009). What we do know is that the dishabituatory stimulus does not have to be strong. Sokolov (1960) showed dishabituation with a stimulus that was lower in intensity in comparison to the habituation stimulus (cited in Barry 2009).

An excellent example of habituation and dishabituation can be seen in the duration of the siphon withdrawal reflex in the sea hare *Aplysia californica* (Kandel and Schwartz 1982). By using a tactile stimulus directed at the siphon, *Aplysia* withdraws its siphon into its body for protection – a response which habituates after repeated presentations. Here Kandel and Schwartz (1982) used an electric shock to the tail as a dishabituation stimulus. The immediate recovery of the response is seen in Fig 4.1 as a large increase in siphon withdrawal reflex duration from 25 seconds to 75 seconds after the delivery of the dishabituating stimulus in the shocked group, with no noticeable difference in the control (not shocked) group.

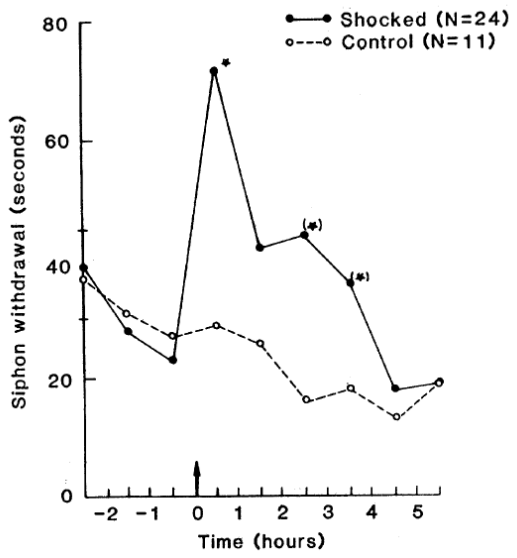

Fig. (4.1). Kandel and Schwartz's **(**1982) example of habituation and dishabituation of the siphon withdrawal reflex duration in the sea hare *Aplysia californica.* A tactile stimulus was used to habituate the siphon and the dishabituation stimulus was an electric shock to the tail, shown here as an arrow at 0 time (hours).

Dishabituation stimuli can be varied, but need to be recognised by the animal as different to the habituation stimulus. For example, leeches (*Hirudo medicinalis*) habituated to electric shock dishabituate with a shock 2V larger than the habituation stimulus (Boulis and Sahley 1988), while jellyfish polyps (*Aurelia aurita*) significantly increase responses to tactile stimulation after a sudden shake (Johnson and Wuensch 1994). Dishabituation stimuli need not occur in the same sensory modality as the habituated stimulus. Although touching *Caenorhabditis elgans* on the head dishabituates it from the tap withdrawal reflex, so does an electric shock (Martinez and Kesner 1998). Similarly, the escape response of *Drosophila* which is habituated to electric shock can be dishabituated with a flash of light (Engle and Wu 1996). Furthermore, Pinsker and colleagues (1970) used a strong tactile stimulus on the neck of *Aplysia* to create dishabituation and not only did the behaviour of the habituated response reoccur, but the readings from the abdominal ganglion recovered in amplitude to match the response before habituation.

In this Chapter I explored Thompson and Spencer's (1966) eighth habituation characteristic (dishabituation) with the salticid *Trite planiceps.* A dishabituation stimulus needs to be perceived as different to the habituation stimulus, which may best be achieved by stimulating a separate sensory modality to the one habituated. Salticids (*Metaphidippus harfordi* and

Metaphidippus aeneolus) habituated to visual stimuli, presented on a rotating drum around salticids that were suspended in the air holding on to a paper ring, dishabituated when the ring was momentarily taken from them prior to the next stimulus presentation (Land 1971). Here, we use a similar concept, but the dishabituation stimulus used was a double puff of air. Air puffs have been successfully used to dishabituate *Drosophila* (Corfas and Dudai 1989), and do not cause the animal any harm. My predictions are: 1. That the presentation of a double air puff will result in recovery of the habituated response. 2. That the salticids which are dishabituated will maintain a higher response rate over the remaining trials than the control salticids.

Materials and methods

Based on the work on *Aplysia* given electric shocks, these experiments introduced a dishabituating stimulus to 'shock' the animal out of its habituated response levels. Here, I used a double puff of air at specified intervals to explore response recovery of the habituated stimulus. Only details pertaining to this particular study will be outlined below. For full methods, refer to Chapter 2.

For these experiments the total number of stimulus presentations, each appearing on-screen for 5 s, was decreased from those in Chapter 3 (160 stimuli) to 80, making the total test time 40 min. The habituating stimulus appeared on the two monitors in alternating fashion, as this eliminated the possibility that each eye received an unequal number of trials. Interstimulus interval was set at 20 s and dishabituating air puffs were given 10 s after stimulus presentation, giving the salticids 10 s to recover before the presentation of the next stimulus and 20 s between the two puffs. The dishabituating air puff stimulus was delivered using a lens cleaning blower brush attached to a clear plastic tube which sat directly behind, and slightly underneath, the salticid. For consistency in the strength of the dishabituating stimulus, the brush was pressed down halfway.

This study consisted of two experiments in which the only difference was that the dishabituating stimulus was given either at regular or irregular intervals. In experiment 1, I tested ten control salticids and ten salticids which were given a double air puff 10 s at specific

intervals, these being prior to stimulus numbers 4, 5, 14, 15, 39, 40, 74 and 75. These intervals were decided because they appear to be low points in the response rate based on the data collected for Chapter 2. In experiment 2, I also used ten controls and an additional ten subjects which received the dishabituating air puffs at irregular intervals. When a test salticid received a dishabituating stimulus was determined by its own responses – if an animal had not responded to the four previous stimulus presentations they were given a double air puff 10 seconds prior to the next stimulus presentation. This was designed to rule out any baseline movement or habituation of the dishabituating stimulus, which may have interfered with the results. The first four instances of animals not responding leading to a dishabituation stimulus were used for analysis so as to be directly comparable to experiment 1, in which there were four dishabituation events.

As in previous tests, responses were video recorded. When presented with a stimulus, the salticid's orienting response causes the polystyrene ball that the salticid is holding to rapidly swivel in the opposite direction, which was counted as a response and scored as 1. Any other movement was not considered a response and was scored as 0. As in Chapters 2 and 3, binary data were analysed using generalized linear mixed models (GLMM) in R (2014). As each salticid was tested over many trials, the family was set as binomial, the link function as Logit and set to the Laplace approximation (Waller *et al.* 2013). Averages for each salticids' responses after each puff of air were analysed using t-tests after checking for normality. Data were averaged for graphing using a moving average of 5 trials and data were fitted with a non-linear exponential function.

Results

In experiment 1, although the salticids decreased their response rate over the course of the trials (Table 4.1, comparison 1) they did not appear to show any change or recovery in response rate to the air puff stimulus when this was presented at pre-defined times (Fig. 4.2). There was a non-significant trend in response rate between the dishabituation and control conditions, in which animals subjected to dishabituation stimulus presentations was marginally less responsive than controls (Table 4.1, comparison 2; Fig. 4.2). The slope of the

non-linear exponential function for dishabituation data was Y = exp (-0.179(x + 5.974)) with an intercept of 0.2059, and for the control data the slope was Y = exp (-0.1804 (x+4.2824)) with an intercept of 0.2249.

A closer look at the data, in which the average response (over the four dishabituating events) of each animal to the visual stimulus immediately succeeding the first puff of air for each of the four dishabituation events was compared with the average response to the visual stimulus succeeding the second puff of air, also showed no significant difference in habituated responses ($t_9 = 0.818$, $p = 0.434$; mean (±SEM) 1st puff response: 0.275 (± 0.069); 2nd puff response: 0.350 (± 0.076)).

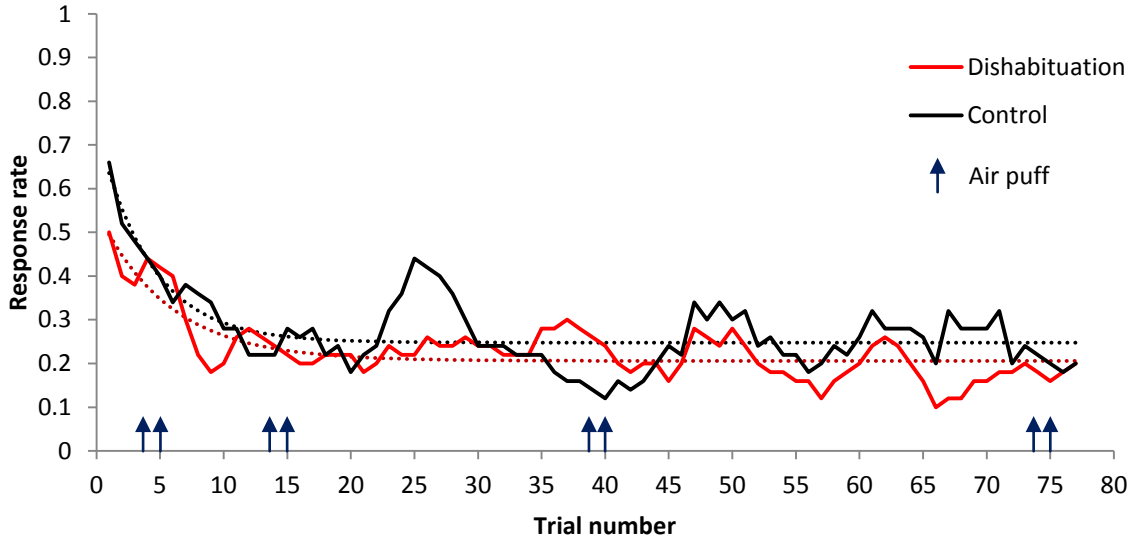

Fig. (4.2) Smoothed average (solid lines) and non-linear exponential function (dotted lines) of habituation to visual stimulus presentation and dishabituation by double air puff at predefined intervals (blue arrows).

Because baseline movement could potentially cause a resumption in response rate and mask dishabituation effects I tested dishabituation again, but instead of presenting the dishabituating stimulus at specified intervals, the salticids were given air puffs after a lack of response to four consecutive stimulus presentations. Out of the four dishabituation events common to all salticids tested, only the first one caused a significantly higher response rate than the control data (Table 4.1, comparison 3), with the overall efficacy of dishabituation apparently dissipating over time, as seen in the functions graphed in Figure 4.3 (see also Table 4.1, comparisons 4-6). The non-linear exponential function slope for dishabituation was Y = exp (-0.12162(x + 12.6586)) with an intercept of 0.2422. The slope for the control data was Y = exp (-8.91796(x + -0.80828) with an intercept of 0.21962.

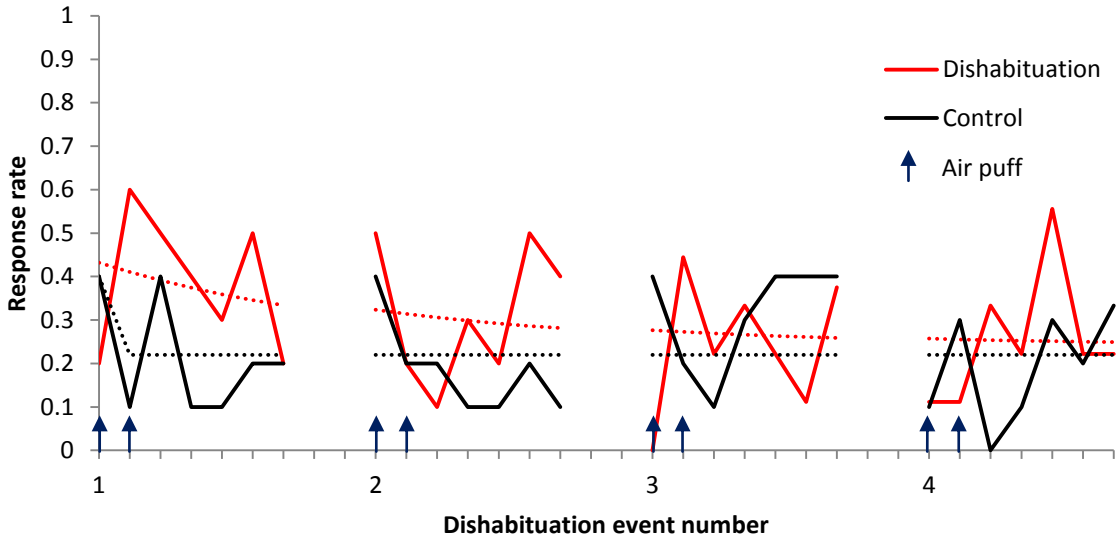

Fig (4.3) Average (solid lines) and non-linear exponential function (dotted lines) of habituation to visual stimulus and dishabituation events (1 to 4) by double air puff at irregular intervals defined by test subjects failing to respond to four consecutive stimulus presentations.

Table (4.1). GLMM results for dishabituation experiments in which the stimulus was presented at regular (R) or irregular (I) time intervals. See text for comparison details. All N=20 except the regular interval experiment, comparison 1 (N=10).

Experiment, comparison	**Test**	**Estimate**	**Standard error**	**z**	***p***
R, 1	Dishabituation response over trials	-0.84	0.094	-8.928	< 0.001
R, 2	Intercept dishabituation vs. control	-0.995	0.099	-10.050	< 0.001
R, 2	Dishabituation vs. control	-0.223	0.116	-1.923	0.055
I, 3	Intercept dishabituation event 1	-1.299	0.291	-4.460	< 0.001
I, 3	Trials dishabituation event 1	0.834	0.381	2.189	0.029
I, 4	Intercept trials dishabituation event 2	-1.4928	0.323	-4.617	< 0.001
I, 4	Trials dishabituation event 2	0.532	0.403	1.747	0.080
I, 5	Intercept trials dishabituation event 3	-0.780	0.257	-3.030	< 0.001
I, 5	Trials dishabituation event 3	0.5191	0.388	1.335	0.181
I, 6	Intercept trials dishabituation event 4	-1.478	0.307	-4.809	< 0.001
I, 6	Trials dishabituation event 4	0.261	0.419	0.625	0.532

Discussion

I predicted recovery of the response rate after the application of the double air puff with *T. planiceps* to be similar to what had been seen previously in *Aplysia* (Kandel and Schwartz 1982) and was surprised that dishabituation did not appear to occur at all in the first experiment. Paradoxically, in experiment 1, the dishabituation treatment salticids appeared to have lower overall response rate than the control salticids. After adjusting the methods, in experiment 2, in order to minimise baseline movement or potential habituation of the dishabituation stimulus, there was only a significant increase in response for the first dishabituation event. Although Thompson and Spencer (1966) established that dishabituation also habituates over repetition, to habituate after one dishabituation stimulus is unusual. The notion that the dishabituation stimulus was not 'strong' enough to disrupt the habituation was dispelled by the recorded responses, which show that almost all salticids showed a startle response by suddenly dropping the ball and waving their legs around (this would have become a jumping escape response if they had not been tethered to the apparatus). Additionally, the dishabituation stimulus does not have to be 'strong' - it just needs to be different (Solokov 1960 cited in Barry 2009). Corfas and Dudai (1989) also used a puff of air as a dishabituation stimulus and found recovery of the response in *Drosophila*. Thus it was unexpected that, despite reacting strongly to the dishabituation stimulus, the salticids then continued ignoring the habituated visual stimulus, especially as the stimuli used were in different sensory modalities (visual and mechanosensory). However, a close look at Fig. 4.3 does suggest that animals did increase their responses (albeit to a considerably lesser extent) even after later dishabituation events. This rapid decrease in response to a sudden air puff is similar to the sudden decrease in response rate to the visual stimulus presented in Chapter 2. This suggests that *T. planiceps* - and potentially salticids in general - may have the ability to rapidly categorise information as non-threatening, which may be an advantage in an environment filled with prey, predators and continuous irrelevant sources of stimuli, including wind. It would be interesting to test concept formation in *T. planiceps* to see if this rapid response decrement is in part due to quickly sorting the stimuli into specific categories. This ability has been recorded in other salticid species (Cross and Jackson 2014), bumblebees (Brown and Sayde 2013) and honeybees (Giurfa *et al.* 2001), and may be a way of economising information in a small brain (Srinivasan 2006).

Andry (1972) found that the arousal level of garter snakes, *Thamnophis radix* and *Thamnophis vagrans*, played a part in their habituation rate, with some becoming so agitated that they exhibited no habituation at all. Although individual differences in habituation rate were apparent in *T. planiceps,* arousal level would have to be very low to not get a dishabituation response. After observing the salticids reacting energetically to the air puffs I would have thought that their arousal level would be relatively high, as they exhibit a violent startle response to the air puffs. The idea that failure of learning may be due to the design of the experiment inflicting low motivation in the animal to respond (Dukas 2008) also seems unlikely. Moving dots across the screen got responses (which decreased over time) during habituation testing. Coupled with the energetic reaction of salticids after the air puffs indicates that the salticid is motivated to respond to both of these stimuli.

It is possible that the salticid has learned that it cannot focus on the stimulus with its high-resolution forward-facing AM eyes and as a result exhibits 'frustration', learned helplessness, or has associated the stimulus with the lack of being able to focus using its AM eyes and gives up. Lack of response due to association has been found in the cuttlefish *Sepia officinalis.* Here cuttlefish learnt by association not to attack a prawn encased in a glass tube. Although the responses using this method have similar characteristics to habituation (e.g., spontaneous recovery of the habituated response after a break from stimulus presentation, and stimulus specificity), the cuttlefish does not dishabituate when the prey stimulus is switched, and therefore the authors classify this behaviour as associative learning (Purdy *et al.* 2006). However, in these cases the animal tends to completely stop responding to all stimuli, while *T. planiceps* maintained a similar habituated response rate throughout the trials, indicating that although this may have been happening it was not acting in isolation.

Because the cellular processes of habituation and dishabituation remain unknown, we do not know if dishabituation is the reversal of habituation or a separate process entirely (Rose and Rankin 2001). It is possible that dishabituation is an alternative process superimposed upon the habituated system and other systems as well (Thompson and Spencer 1966). This becomes problematic because Thompson and Spencer's (1966) characteristics of habituation states that recovery of a response after dishabituation indicates that the process is indeed habituation. Therefore, dishabituation has become an indicator of true habituation and not

another form of response decrement. Taken as true, this definition makes interpretation of my results difficult, because although *T. planiceps* follow the first and fourth characteristics of habituation, the eighth characteristic of dishabituation rendered only a small result, and then only in experiment two. To further understand dishabituation in *T. planiceps* I would ideally like to try using another dishabituation stimulus, perhaps vibration or mechanical stimulation by gently touching the salticid with a paintbrush.

Rose and Rankin (2001) discovered that *C. elgans* exhibited differences in the rate of spontaneous recovery depending on which interstimulus interval was given during habituation testing. For short ISI, of 2 seconds, the spontaneous recovery of the response was rapid and near completely restored after 30 minutes without testing. However, with an ISI of 60 s the response had barely recovered after 30 minutes. Rose and Rankin (2001) suggest that this phenomenon is an important characteristic of habituation as this trend in spontaneous recovery is the opposite of what would be expected for sensory adaptation or fatigue. The authors also suggest that spontaneous recovery may be preferable to dishabituation in defining habituation, as the mechanism for dishabituation is unknown. To be completely concrete in ruling habituation in or out, this method would need to be done with *T. planiceps*, which have already been shown to exhibit differences in response rate to different interstimulus interval values (Chapter 3). Future tests should be extended to explore the salticids' spontaneous recovery at multiple time periods after testing using different ISI.

In summary, the results obtained from dishabituation tests were not consistent between experiments. In particular, for tests in which the dishabituation stimulus was given a predetermined times, responses did not appear to increase at all, whereas there was some increase in response rate when the dishabituation puffs were presented to animals after a certain time of no responses. It is seems unlikely that habituation to the dishabituation stimulus (especially considering that I used a double puff of air - perhaps leading the animal to habituate to the second puff) led to my results, as a comparison between responses to the habituated stimulus did not differ between the first and second puff of air. Had the animals rapidly habituated to the air puff, I would have expected to see an increase in responses after the first puff, with a decrease in response to the visual stimulus after the second puff. The most likely explanation may simply be that the animals were in fact too startled by the stimulus, leading to a freezing response, as discussed in Chapter 3.

References

Andry, M. L. & Luttges, M. W. 1972. Neural habituation in garter snakes. *Physiology & Behaviour*, **9,** 107-111**.**

Barry, R. J. 2009. Habituation of the orienting reflex and the development of Preliminary Process Theory. *Neurobiology of Learning and Memory*, **92**, 235-242.

Boulis, N. M. & Sahley, C. 1988. A behavioural analysis of habituation and sensitization of shortening in the semi-intact leech. *The Journal of Neuroscience*, **8**, 4621-4627.

Brown, M. F. & Sayde, J. M. 2013. Same/different discrimination by bumblebee colonies. *Animal Cognition*, **16**, 117-125.

Cross, F. R. & Jackson, R. R. 2014. Specialised use of working memory by *Portia africana*, a spider-eating salticid. *Animal Cognition*, **17**, 435-444.

Dukas, R. 2008. Evolutionary Biology of Insect Learning. *Annual Review of Entomology*, **53**, 145-160.

Engel, J. E. & Wu, C.-F. 1996. Altered habituation of an identified escape circuit in *Drosophila* memory mutants. *The Journal of Neuroscience*, **16**, 3486-3499.

Geer, J. H. 1966. Effect of interstimulus intervals and rest-period length upon habituation of the orienting response. *Journal of Experimental Psychology*, **72**, 617.

Giurfa, M., Zhang, S., Jenett, A., Menzel, R., and Srinivasan, M.V. 2001. The concepts of 'sameness' and 'difference' in an insect. *Nature*, **410**, 930–933.

Glanzman, D. L. 2009. Habituation in Aplysia: the Cheshire cat of neurobiology. *Neurobiology of Learning and Memory*, **92**, 147-154.

Groves, P. M. & Thompson, R. F. 1970. Habituation: a dual-process theory. *Psychological Review*, **77**, 419.

Holmes, S. J. 1912. Phototaxis in the sea urchin, *Arbacia punctulata*. *Journal of Animal Behavior*, **2**, 126.

Humphrey, G. 1933. *The nature of learning in its relation to the living system*. New York: Harcourt, Brace.

Johnson, M. C. & Wuensch, K. L. 1994. An investigation of habituation in the jellyfish *Aurelia aurita*. *Behavioural and Neural Biology*, **61**, 54-59.

Kandel, E. R. & Schwartz, J. H. 1982. Molecular biology of learning: modulation of transmitter release. *Science*, **218**, 433-443.

Klingner, C. M., Hasler, C., Brodoehl, S. & Witte, O. W. 2014. Excitatory and inhibitory mechanisms underlying somatosensory habituation. *Human Brain Mapping*, **35**, 152-160.

Land, M. 1971. Orientation by jumping spiders in the absence of visual feedback. *Journal of Experimental Biology*, **54**, 119-139.

Mackworth, J. F. 1968. Vigilance, arousal, and habituation. *Psychological Review*, **75**, 308.

Martinez, J. L., & Kesner, R.P. 1998. *Neurobiology of Learning and Memory*. San Diego.: Academic Press Inc.

Mazur, J. E. 2006. *Learning and Behaviour*, Sixth Edition. Upper Saddle River, NJ.: Pearson, Prentice Hall.

Pinsker, H., Kupfermann, I., Castellucci, V. & Kandel, E. 1970. Habituation and dishabituation of the GM-withdrawal reflex in *Aplysia*. *Science*, **167**, 1740-1742.

Purdy, J. E., Dixon, D., Estrada, A., Peters, A., Riedlinger, E. & Suarez, R. 2006. Prawn-in-a-tube procedure: Habituation or associative learning in cuttlefish? *The Journal of General Psychology*, **133**, 131-152.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C.-F. & Thompson, R. F. 2009. Habituation revisited: An updated and revised description of the behavioural characteristics of habituation. *Neurobiology of Learning and Memory*, **92**, 135-138.

Rose, J. K. & Rankin, C. H. 2001. Analyses of habituation in *Caenorhabditis elegans*. *Learning & Memory*, **8**, 63-69.

Srinivasan, M.V. 2006. Honeybee vision: in good shape for shape recognition. *Current Biology*, **16**, R58–R60.

Thompson, R. F. & Spencer, W. A. 1966. Habituation: a model phenomenon for the study of neuronal substrates of behaviour. *Psychological Review*, **73**, 16.

Waller, B., Warmelink, L., Liebal, K., Micheletta, J. & Slocombe, K. 2013. Pseudoreplication: a widespread problem in primate communication research. *Animal Behaviour*, **86**, 483-488.

Wood, D. C. 1988. Habituation in *Stentor*: produced by mechanoreceptor channel modification. *The Journal of Neuroscience*, **8**, 2254-2258.

Chapter 5. Discussion.

Do jumping spiders habituate to repetitive stimuli?

The ability to discriminate a potential predator from an innocuous movement is crucial for survival. Habituation allows the animal to filter out repetitive *irrelevant* stimuli (Rose and Rankin 2001), such as objects moving from waves or wind. However, for a response decrement to be considered the process of habituation it must fit within the ten characteristics first categorised by Thompson and Spencer (1966), Groves and Thompson (1970) and later edited by Rankin and collegues (Rankin *et al.* 2009). In my research I tested three of these characteristics with the salticid *Trite planiceps*:

1. The repeated application of a stimulus results in a progressive decrease in response to that stimulus,
4. More frequent stimulation results in a faster or more pronounced response decrement and also results in more rapid spontaneous recovery,
8. Once the animal has habituated to the stimuli the presentation of a different stimulus results in the recovery of the habituated response. This is called dishabituation.

Habituation of the orientation response in salticids has rarely been studied. Land (1971) found that when the salticids *Metaphidippus harfordi* and *Metaphidippus aeneolus* experienced repetitive visual stimuli in the same region of the retina, they were faster at habituating than salticids that experienced stimuli in a new region of the retina.
I explored habituation of the orientation response of the salticid *Trite planiceps*. In Chapter 2 I found that the response rate of *T. planiceps* rapidly declined in the first five to ten trials, and then maintained the same response rate over the rest of the trials. The salticids showed a lower response rate when stimuli appeared on one side of the two monitors compared to stimuli appearing randomly on both monitors. This was likely due to the random nature of the stimuli making them more unpredictable for the salticid. In Chapter 3 I tested two interstimulus intervals (ISI) of 10 and 20 seconds during habituation testing on *T. planiceps.* I found that the interstimulus interval had an effect in initial decrement which was maintained through the remaining trials. Then in Chapter 4 I tested dishabituation with *T. planiceps* with

a double air puff. I expected a dramatic change in response rate similar to other dishabituation results across other invertebrates and was surprised to get only a small reaction.
Chapter 2 and Chapter 3 fairly strongly suggest habituation had occurred with *T. planiceps*, but Chapter 4 was not clear cut in its results only suggesting the dishabituation had happened. To be completely confident I would need to do further research on dishabituation with methods I have explained in great detail in Chapter 4; such as applying a different dishabituation stimulus. Thompson and Spencer's (1966) ten characteristics of habituation states that recovery of a response after dishabituation indicates that the process is indeed habituation. Therefore, dishabituation has become an indicator of true habituation and not another form of response decrement. Because *T. planiceps* did not show a clear distinction of recovery after dishabituation this means that the response decrement in Chapter 2 cannot be considered habituation until we get good results from dishabituation. Thompson and Spencer (1966) also state that habituation can usually be distinguished from other decrements because it is reversible; habituated responses show spontaneous recovery. That is when the stimulus is removed for a short period of time and then retested, during the retesting the response rate should jump back above the previously habituated rate. Before making any conclusions this would need to be tested in *T. planiceps*. Although some conditions would be difficult to test with salticids, I would ideally like to test the remaining ten characteristics of Thompson and Spencer's (1966) habituation criteria to fully understand the process of habituation in *T. planiceps*.

The complications of a simple theory

Habituation protects the organisms' nervous system from being flooded with excessive information (Klingner *et al*. 2014), by filtering out repetitive irrelevant information. However, the cellular process that regulates what is ignored and what is attended to is still being uncovered somewhat impatiently (Glanzman 2009). The problem arises because habituation is considered the simplest form of learning (Christensen *et al.* 2008), as it is not associated with another stimulus just the same stimulus repeated many times. Therefore, to properly understand more complex forms of learning such as associative learning, habituation must first be completely understood (Rose and Rankin 2001). However, the idea of habituation being a straightforward process is somewhat hopeful, and despite over a century of work on the subject (Thompson 2009) remains a mystery. This is partially because although non-associative learning is the simplest stimuli with which to test animals, the actual process of habituation requires the neural system to constantly assess incoming stimuli and

then filter out those that are not important, while retaining important stimuli. The number of neuronal elements, the complexity of the circuits which receive sensory input to then communicate with the central nervous system and the difficulty of matching behaviour with molecular changes combine to make this research challenging (Osborn *et al.* 1973). Therefore, habituation still contains cellular mechanisms which are yet to be revealed (Rankin *et al.* 2009). Additionally because the cellular processes of habituation and dishabituation remain unknown, we do not know if dishabituation is the reversal of habituation or a separate process entirely (Rose and Rankin 2001). It is also possibly that habituation is due to a number of different processes overlapping rather than a single process, this can be seen when testing interstimulus interval with species which exhibit differences in memory retention between 'long' and 'short term' habituation and spontaneous recovery (Rose and Rankin 2001). It also worth noting the added complication that learning processes such as habituation do not act entirely in isolation; other learning mechanisms may also be operating concurrently (Stiles and Ghosh 1997), such as associative learning.

What is at least reassuring is that previous habituation research has encompassed a wide range of animal phyla, from the protozoan *Stentor* (Wood 1988), nematodes (Rose and Rankin 2001), insects (Engel and Wu 1996), arachnids (Land 1971), molluscs (Long *et al.* 1989) and on to vertebrates (Schloegl *et al.* 2007) and even humans (Sokolov 1960; cited in Barry 2009). This wide range of habituation supports the hypothesis that habituation is critical for survival, as it has not been altered significantly through evolution, and may consist of cellular mechanisms which are conserved across phylogenies (Rose and Rankin 2001). Furthermore, although we are distantly related, knowledge of invertebrate nervous systems could provide answers to the problems of our own nervous systems (Edwards *et al.* 1999). Invertebrate nervous systems generally contain only several thousand (or hundred thousand) neurons (as opposed to billions in many vertebrates) making them easier candidates for research into learning and memory than vertebrates (Martinez and Kesner 1998). Completely understanding the mechanisms of habituation could have far reaching implications with human mental health research, as disturbances in habituation have been linked to disorders such as schizophrenia, aging, autism and substance abuse (Artigas 2012). Research using invertebrates may provide the necessary platform from which to understand how nervous systems facilitate and change due to learned behaviour (Martinez and Kesner 1998), which can then be applied to other fields of research.

Habituation is linked to attention research

From the optic nerve alone the number of incoming stimuli is estimated to be approximately 10^7- 10^8 bits per second. Therefore, instead of attempting to process all of the sensory input, attention filters out what is relevant and irrelevant (Itti and Koch 2001). Broadbent (1958) proposed the filter theory based on the concept that all sensory information reaches some sort of bottleneck (attention) where the person chooses the message to be processed based on the characteristics of the information. The bottleneck of visual information processing can be best demonstrated with the classic video by Simons and Chabris (1999). (The original video can be found here: https://www.youtube.com/watch?v=vJG698U2Mvo.) In this video the watcher is required to count the basketball passes of the players with either white or black clothes, but the people are constantly moving so it is a demanding task. At a certain time a person in a gorilla suit walks right between the players. The people who are passively watching will notice the gorilla immediately, 67% of people counting the black clothes will notice the gorilla, but those focussed on the white clothes will not 'see' the man in the gorilla suit until it is pointed out to them (8% noticed). This is because these people have selected the white as the characteristic of information to be brought to attention and have filtered out the remaining information (Simons and Chabris 1999). The concept of the bottleneck of attention follows the same logic as habituation, with the main difference being that attention fixates on the relevant stimuli and moving to other stimuli, and habituation fixates on the one stimulus and then filters it out as irrelevant. The process of habituation in attention research is barely mentioned, but habituation is interesting from the viewpoint of attention because the stimulus that initially elicits a response eventually, after repetition, does not. An example of this is provided in Kahneman's (1973) book *Attention and Effort*, where the author describes an experiment where a dog is exposed to a tone at regular intervals and does not respond, but when a tone of a different pitch is added the dog moves its eyes, pricks its ears and catches its breath. Although in this book this example is used to explain attention, this sort of experiment mimics auditory habituation and dishabituation via the new tone. Therefore, understanding the mechanisms of habituation may further uncover the limitations of sustained attention in psychological research.

Understanding the limits of human performance is important as these limitations have been the cause of catastrophes in aviation and other areas. Consequently, understanding the limits of human attention may help develop methods or techniques that may prevent or minimise such catastrophes (Pashler *et al.* 2001). One known phenomenon of the limitations of

attention is vigilance decrement. Vigilance decrement is the inability to maintain attention on a task over long periods of time (Davies and Parasuraman 1982). As the time on task increases, participants gradually begin slowing their responses to stimuli or missing them entirely (Mackworth (1948, 1950); Head and Helton 2012). Research on vigilance decrement began during the Second World War, after Mackworth (1948, 1950) noticed that observers were unable to maintain a high level of detection performance on a radar screening task as they remained on watch. While vigilance decrement primarily was studied out of interest in military operations (Helton and Russell 2012), it has caught the attention of educators (Risko *et al*. 2012), life guards (Fenner *et al*. 1999), and long-distance drivers (Verster and Roth 2012). This is due to the high probability of accidents, ranging from mild to severe, that are often caused by vigilance failures from human operators (Warm *et al*. 2008). Once again, the mechanism of vigilance decrement, like habituation, is not yet understood at the cellular level (Dukas and Clark 1995). It seems likely that in order to do so, we should be focussing our attention to building a complete understanding of simple learning processes, such as habituation, with animals with simpler nervous systems before we can begin to completely understand complex phenomenon like attention and vigilance decrement.

References

Artigas, A. A., Sansa, J. & Prados, J. 2012. Distractor effects upon habituation of complex stimuli. *Behavioural Processes*, **90**, 204-209.

Barry, R. J. 2009. Habituation of the orienting reflex and the development of Preliminary Process Theory. *Neurobiology of Learning and Memory*, **92**, 235-242.

Broadbent, D. E. 1958. *Perception and Communication*. New York: Oxford University Press.

Christensen, J. W., Zharkikh, T. & Ladewig, J. 2008. Do horses generalise between objects during habituation? *Applied Animal Behaviour Science*, **114**, 509-520.

Davies, D. R., & Parasuraman, R. 1982. *The psychology of vigilance.* London: Academic Press.

Dukas, R. & Clark, C. W. 1995. Sustained vigilance and animal performance. *Animal Behaviour*, **49**, 1259-1267.

Engel, J. E. & Wu, C.-F. 1996. Altered habituation of an identified escape circuit in *Drosophila* memory mutants. *The Journal of Neuroscience*, **16**, 3486-3499.

Edwards, D. H., Heitler, W. J. & Krasne, F. B. 1999. Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. *Trends in Neurosciences*, **22**, 153-161.

Fenner, P., Leahy, S., Buhk, A. & Dawes, P. 1999. Prevention of drowning: visual scanning and attention span in lifeguards. *Journal of Occupational Health and Safety Australia and New Zealand*, **15**, 61-66.

Groves, P. M. & Thompson, R. F. 1970. Habituation: a dual-process theory. *Psychological Review*, **77**, 419.

Head, J. & Helton, W. S. 2012. Natural scene stimuli and lapses of sustained attention. *Consciousness and Cognition*, **21**, 1617-1625.

Helton, W. S. & Russell, P. N. 2012. Brief mental breaks and content-free cues may not keep you focused. *Experimental Brain Research*, **219**, 37-46.

Itti, L. & Koch, C. 2001. Computational modelling of visual attention. *Nature Reviews Neuroscience*, **2**, 194-203.

Kahneman, D. 1973. *Attention and Effort* (p. 246). Englewood Cliffs, NJ: Prentice-Hall.

Klingner, C. M., Hasler, C., Brodoehl, S. & Witte, O. W. 2014. Excitatory and inhibitory mechanisms underlying somatosensory habituation. *Human Brain Mapping*, **35**, 152-160.

Land, M. 1971. Orientation by jumping spiders in the absence of visual feedback. *Journal of Experimental Biology*, **54**, 119-139.

Long, T. M., Hanlon, R. T., Maat, A. T. & Pinsker, H. M. 1989. Non-associative learning in the squid *Lolliguncula brevis* (Mollusca, Cephalopoda). *Marine & Freshwater Behaviour & Physiology*, **16**, 1-9.

Mackworth, N. 1948. The breakdown of vigilance during prolonged visual search. *Quarterly Journal of Experimental Psychology*, **1**, 6-21.

Mackworth, N. 1950. *Researches on the measurement of human performance*. Reprinted in H. W. Sinaiko (Ed.), *Selected Papers on Human Factors in the Design and Use of Control Systems*. 1961. Dover Publications, New York, 174-331.

Osborn, D., Blair, H. J., Thomas, J. & Eisenstein, E. 1973. The effects of vibratory and electrical stimulation on habituation in the ciliated protozoan, *Spirostomum ambiguum*. *Behavioral Biology*, **8**, 655-664.

Pashler, H., Johnston, J. C. & Ruthruff, E. 2001. Attention and performance. *Annual Review of Psychology*, **52**, 629-651.

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C.-F. & Thompson, R. F. 2009. Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. *Neurobiology of Learning and Memory*, **92**, 135-138.

Risko, E. F., Anderson, N., Sarwal, A., Engelhardt, M. & Kingstone, A. 2012. Everyday attention: variation in mind wandering and memory in a lecture. *Applied Cognitive Psychology*, **26**, 234-242.

Rose, J. K. & Rankin, C. H. 2001. Analyses of habituation in *Caenorhabditis elegans*. *Learning & Memory*, **8**, 63-69.

Schloegl, C., Kotrschal, K. & Bugnyar, T. 2007. Gaze following in common ravens, *Corvus corax*: ontogeny and habituation. *Animal Behaviour*, **74**, 769-778.

Simons, D. J. & Chabris, C. F. 1999. Gorillas in our midst: Sustained inattentional blindness for dynamic events. *Perception-London*, **28**, 1059-1074.

Stiles, B. W. & Ghosh, J. 1997. Habituation based neural networks for spatio-temporal classification. *Neurocomputing*, **15**, 273-307.

Thompson, R. F. 2009. Habituation: A history. *Neurobiology of Learning and Memory*, **92**, 127-134.

Thompson, R. F. & Spencer, W. A. 1966. Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychological Review*, **73**, 16.

Verster, J. C., Bervoets, A. C., de Klerk, S. & Roth, T. 2014. Lapses of attention as outcome measure of the on-the-road driving test. *Psychopharmacology*, **231**, 283-292.

Warm, J. S., Parasuraman, R. & Matthews, G. 2008. Vigilance requires hard mental work and is stressful. *Human Factors: The Journal of the Human Factors and Ergonomics Society*, **50**, 433-441.

Wood, D. C. 1988. Habituation in *Stentor*: produced by mechanoreceptor channel modification. *The Journal of Neuroscience*, **8**, 2254-2258.